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BACTERIAL ACCUMULATION BY THE OYSTER, CRASSOSTREA VIRGINICA, ON THE GULF COAST

C. B. Kelly, et al

Robert A. Taft Sanitary Engineering Center
Cincinnati, Ohio

1960

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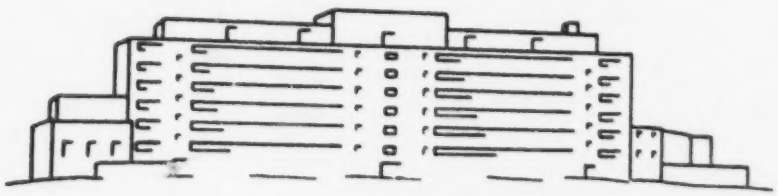

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BACTERIAL ACCUMULATION BY THE OYSTER, CRASSOSTREA VIRGINICA, ON THE GULF COAST

C. B. Kelly, W. Arcisz, M. W. Presnell

Shellfish Sanitation Laboratory
Milk and Food Research Program

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Statistical Services

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Public Health Service
Bureau of State Services
Division of Engineering Services

Robert A. Taft Sanitary Engineering Center
Cincinnati 26, Ohio

1960

I

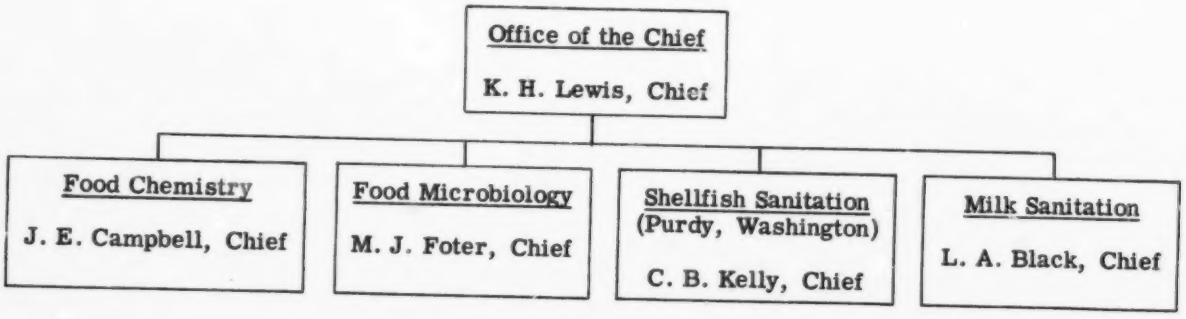
MILK AND FOOD RESEARCH

The Milk and Food Research activities of the Sanitary Engineering Center develop experimental evidence and provide technical services in support of both the Milk and Food and the General Engineering Programs of the Division of Engineering Services, as well as the Research Branch of the Division of Radiological Health. In addition to publication of research findings, the practical applications are presented directly to public health workers in seminars and specialized courses offered by the training activities of the Sanitary Engineering Center or the Communicable Disease Center.

Emphasis is given to experimental work and collaborative studies on public health problems associated with milk, shellfish, and other perishable foods. Specialists in microbiology, biochemistry, and food technology conducted laboratory and field investigations to improve methodology, evaluate health hazards, and determine the sources of contimaination to which these foods may be subjected during production, processing, storage, and preparation for sering. These studies are oriented to serve the needs of State and local health agencies and industry in preventing the occurence of foodborne illnesses. Fact-finding, interpretation of observations, and application of new knowledge for the protection of the consumer's health are the principal goals.

For administrative convenience, the staff is organized into four units as shown below. The Shellfish Sanitation laboratory occupies facilities provided by the Wasnington State Health Department near an oyster-growing estuary of Puget Sound. The Office of the Chief and the other three units are located at the Sanitary Engineering Center, 4676 Columbia Parkway, Cincinnati 26, Ohio.

Milk and Food Research



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BACTERIAL ACCUMULATION BY THE OYSTER, *CRASSOSTREA VIRGINICA*, ON THE GULF COAST

C. B. Kelly, W. Arcisz and M. W. Presnell

Shellfish Sanitation Laboratory*
Milk and Food Research Program

SUMMARY

A series of laboratory experiments were conducted at Pensacola, Florida to determine the rate of accumulation of bacteria by the eastern oyster, Crassostrea virginica. These studies involved the determination of the coliform level attained by oysters in laboratory flats furnished flowing sea water artificially polluted by the continuous addition of suspensions of coliform organisms. The tests were conducted simultaneously at two levels of bacterial density of the water, which correspond to the coliform MPN limits set for moderately polluted and approved shellfish growing areas. A third flat, furnished with coliform-free sea water, was included as a control.

Definite seasonal variations in rate of accumulation were observed. The highest oyster-water coliform ratio, which averaged approximately 16 to 1 in five experiments, was found to occur in the late fall immediately after a rapid decline in water temperature from 29°C to 20°C and below. The ratios continued high for a period of approximately 6 weeks. On approach to water temperatures of 15°C the rate of accumulation reduced and continued at the low level for two months. At water temperatures above 25°C the rate of bacterial accumulation was also found to be lower than at water temperatures between 15 and 20°C.

The seasonal changes observed in these studies correlate quite well with the results obtained in two other investigations conducted at this same location. In one, a sharp increase in rate of pumping was noted following the same pattern of decline in water temperature as preceded our sharp increase in rate of bacterial accumulation. Lowered rates of pumping were found at water temperatures above 25°C and below 15°C. Another investigation demonstrated a sharp increase in growth rate of oysters at similar water temperatures and seasons and a reduced rate of growth at higher temperatures.

The rate of accumulation was found to vary quite widely within an apparently similar temperature or seasonal condition. Ranges of as much as 30 to 1 in oyster-water coliform ratios were found, sometimes within a few hours of each other. To what extent these variations are due to individual differences or to sudden changes in the characteristic of the sea water we did not determine. Variations in pumping rate of this amplitude have been reported, also within short periods of time.

During preliminary experiments in which the experimental system was under evaluation, it was found that the feeding activity of oysters was influenced by chlorination and dechlorination of the sea water. The number of oysters showing activity in chlorinated water was significantly less than that of oysters receiving untreated water. Furthermore, it was noted that the degree of activity was reduced in oysters receiving chlorinated and dechlorinated water. Ultraviolet treatment of sea water proved to be an effective means of destroying coliform organisms. Ultraviolet treatment of sea water was demonstrated to produce no factor which inhibited the feeding activity of oysters.

*Since completion of this work the Laboratory has been moved and is presently located near Gig Harbor, Washington.

INTRODUCTION

In the sanitary control of shellfish, it has long been an established practice to judge the sanitary quality of a shellfish growing area by a comprehensive sanitary survey and bacteriological examination of the water to determine the potential sources of pollution and the probable presence of residual quantities of dangerous pollution of human or animal origin that might be present in the harvesting area. Examination of shellfish as harvested cannot be relied on as an accurate means of assessing the sanitary quality of a growing area. Wide fluctuations have been observed in the coliform MPN* of oysters as harvested and in some instances these fluctuations could not be correlated with changes in bacterial quality of the water.

This report describes laboratory experiments which were designed to study the water-shellfish relationships at controlled levels of pollution in order to determine the influence of temperature, season, and other factors on the rate of bacterial accumulation by the eastern oyster, Crassostrea virginica.

That shellfish, particularly oysters, vary in physiological activity is a well established fact. There are many reports (1-6) which demonstrate that the activity of oysters is influenced greatly by temperature, and other reports (7, 8) mention that the activity is influenced by certain organic constituents of the overlying water.

Our experiments were conducted near Pensacola, Florida, using as the source of water an arm of Pensacola Bay somewhat removed from the influence of pollution by the city proper. The experiments involved subjecting oysters to a continuous flow of sea water to which had been added, under controlled conditions, suspensions of three representative coliform types. Samples of water and oysters were collected simultaneously and at regular intervals. In each experiment separate lots of oysters were submitted to water of three coliform levels: flat "A" was fed water with a coliform level of approximately 1,000 MPN per 100 ml., flat "B" with water at approximately 100 MPN. Flat C was a control, receiving sea water without added pollutant.

*Most Probable Number

Part I

METHODS AND EQUIPMENT

EXPERIMENTAL DESIGN

The experimental system consisted essentially of four component parts: The salt water supply, the water treatment system, the polluting system and the experimental oyster flats.

Sea Water Supply

The sea water was obtained from Santa Rosa Sound immediately off-shore from the laboratory. Duplicate intakes and pumping equipment were provided, each intake approximately 60 feet long, extending into a 15-foot depth of water. The water was pumped by means of centrifugal pumps into a 55 gallon drum provided with an overflow. The drum with the overflow served as a constant head device. Water for the experiment was taken from near the bottom of the drum and the flow regulated by means of an all iron gate valve. A flow diagram of the water treatment system, the polluting system and the experimental oyster flats is shown in Figure 1.

The water flowed from the drum through four germicidal lamp units, open troughs, 8 inches wide, 36 inches long and 6 inches deep, connected in series. Depth of water in the trough was maintained at 2-1/2 inches. Each trough was provided with a logitudinal baffle 2-1/2 inches high, placed on the center line of the trough. Flow was between diagonally opposite corners. A semicylindrical arch shaped reflector set on top of each trough was lined with a sheet of alzak specular finish aluminum and provided with two 30 watt germicidal lamps 30 inches long. With a water flow of 40 liters per minute the theoretical detention time in the system was 70 seconds and minimum detention as timed by fluorescein dye was (45) seconds.

A standpipe with overflow was installed at the far end of the distribution system to serve as a constant head device. Water flow to the individual flats was controlled by all iron gate valves.

EXPERIMENTAL FLATS

The experimental flats were wooden troughs 40 inches wide, 80 inches long and ten inches deep, constructed of 2 inch cypress and painted with asphalt varnish. Water entered the trough through a distributing manifold constructed of plexiglass tubing 1-3/4 inches in diameter. An overflow weir, 3-1/2 inches high at the discharge end of the trough established the water level. Two longitudinal baffles, extending from end to end of the trough, were provided to reduce circular flow.

The functional portion of the trough had a capacity of 180 liters. At the usual flow of 12 liters per minute, calculated flow time through the flats was 15 minutes.

POLLUTION SYSTEM

The polluting agent used in these experiments was a suspension three coliform types, Escherichia coli, E. coli intermediate and Aerobacter aerogenes, in phosphate buffered dilution water. The suspensions of bacteria were obtained from 24 hour hear infusion agar slants of constant surface area. The bacteria were washed from the slants and brought up to 100 ml with phosphate buffered dilution water. Experience demonstrated that with this technique we could obtain fairly constant densities in the polluting agent. However, the actual density of the suspension was determined by 24 hour plate counts on tryptone glucose extract agar. The supply of polluting agent was stored in either of two aquaria and placed in insulated boxes. In order to prevent reduction in coliform organisms during the 12 to 14 hours of use, polluting agent was maintained at a temperature of 10°C. Refrigeration was accomplished by passing cooled ethylene glycol solution through coils of aluminum tubing immersed in the aquaria. The suspension was kept constantly stirred by means of an electrically operated stirring device. A thermo-regulator, immersed in the polluting agent, actuated a pump which circu-

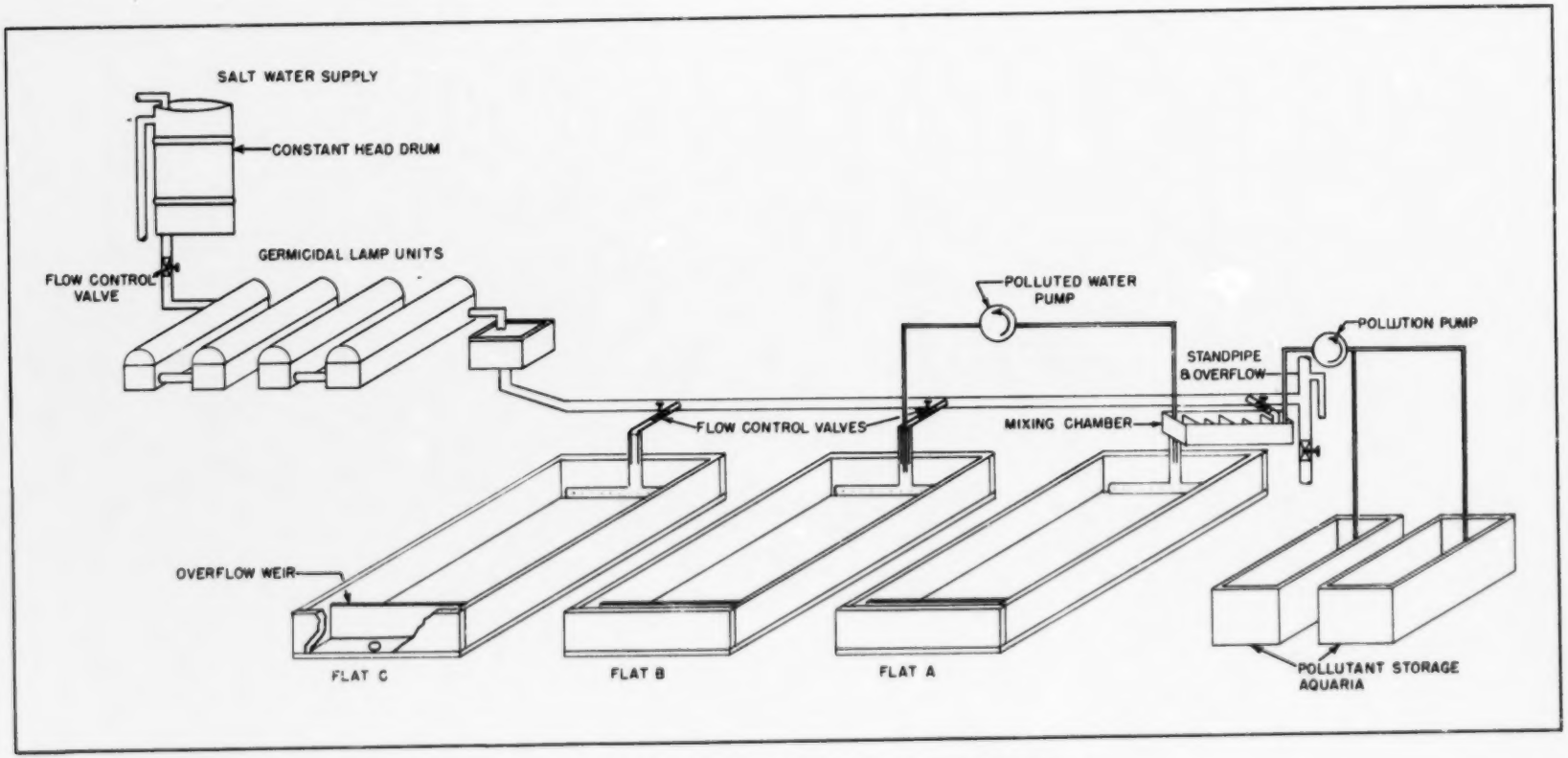

Figure 1. DIAGRAM OF EXPERIMENTAL EQUIPMENT

TABLE 1. TYPICAL OPERATING RECORD

Date	Time	Sea water flow Liters/min			Pollution flow ml/min	
		Flat A	Flat B	Flat C	Pump A	Pump B
11/15/55	8:25 AM	13	12	12	53	815
	6:00 PM	13	13	12	52	830
11/16/55	7:00 AM	12	13	12	53	840
	10:00 AM	12	13	12	52	840
	2:00 PM	12	12	12	50	830
	6:00 PM	12	12	12	52	830
	9:00 PM	12	12	12	51	820
11/17/55	7:00 AM	12	10.4	11	52	820
	2:00 PM	12	12	12	50	820
	6:00 PM	12	12	10	50	810
	9:00 PM	12	13	11	50	820
11/18/59	7:00 AM	12	12	10	50	810
	10:00 AM	12	12	10	50	800
	2:00 PM	12	12	10	50	850
	6:00 PM	12	12	10	50	800
	9:00 PM	12	12	10	50	800

lated the refrigerent through the coils and thus controlled the temperature.

The polluting agent was proportionated with the water entering flat "A" by means of a Brittingham pump capable of a wide range of discharges, but for this application was maintained at a flow of 50 ml per minute. This flow and the concentration of organisms in the pollution reservoir were calculated to maintain the coliform level of the water entering flat "A" at an MPN of approximately 1,000 per 100 ml. The Brittingham pump is illustrated in figure 2.

A portion of the polluted water was drawn from the discharge end of the mixing chamber by means of a larger Brittingham pump, the discharge rate of the pump being adjusted to proportionate sufficient quantity of flat "A" water with UV treated sea water to produce a coliform MPN density of 100 per 100 ml entering flat "B". Mixing was accomplished in the distributing manifold. Control flat C received no polluting agent.

A constant flow was necessary to maintain the desired pollution levels. After a very short period of "working-in" the variation in flow of the Brittingham pumps was not greater than five percent.

During the course of the experiments, records were kept of the water flows into each of the experimental flats, the flow of the two pollution pumps "A" and "B", and temperature of the water in the flats as measured by a recording thermometer. Flow records for a typical experiment are shown in Table 1.

EXPERIMENTAL OYSTERS

The oysters used in these experiments were obtained from beds in Bon Secour Bay, Alabama, the nearest commercial source. Most were taken from a natural reef known as Bon Secour Reef. They were of small commercial size, averaging 2-1/2 to 3-1/2 inches from hinge to bill. The oysters were obtained from the diggers on arrival at the dock, sorted for size and transported to the laboratory. Time of transportation to the laboratory was usually less than two hours. On arrival at the laboratory they were immediately placed in a tank of flowing sea water and so maintained until they were prepared for use in the experiment.

Four to seven days prior to the start of the experiment the oysters were scraped and brushed to remove extraneous growth of algae, barnacles and other adhering molluscs. They were then transferred to the experimental flats. From then until the start of the experiment, the flats were flushed and sanitized daily. The flats were first hosed with fresh water to wash out accumulated fecal discharges and then filled with

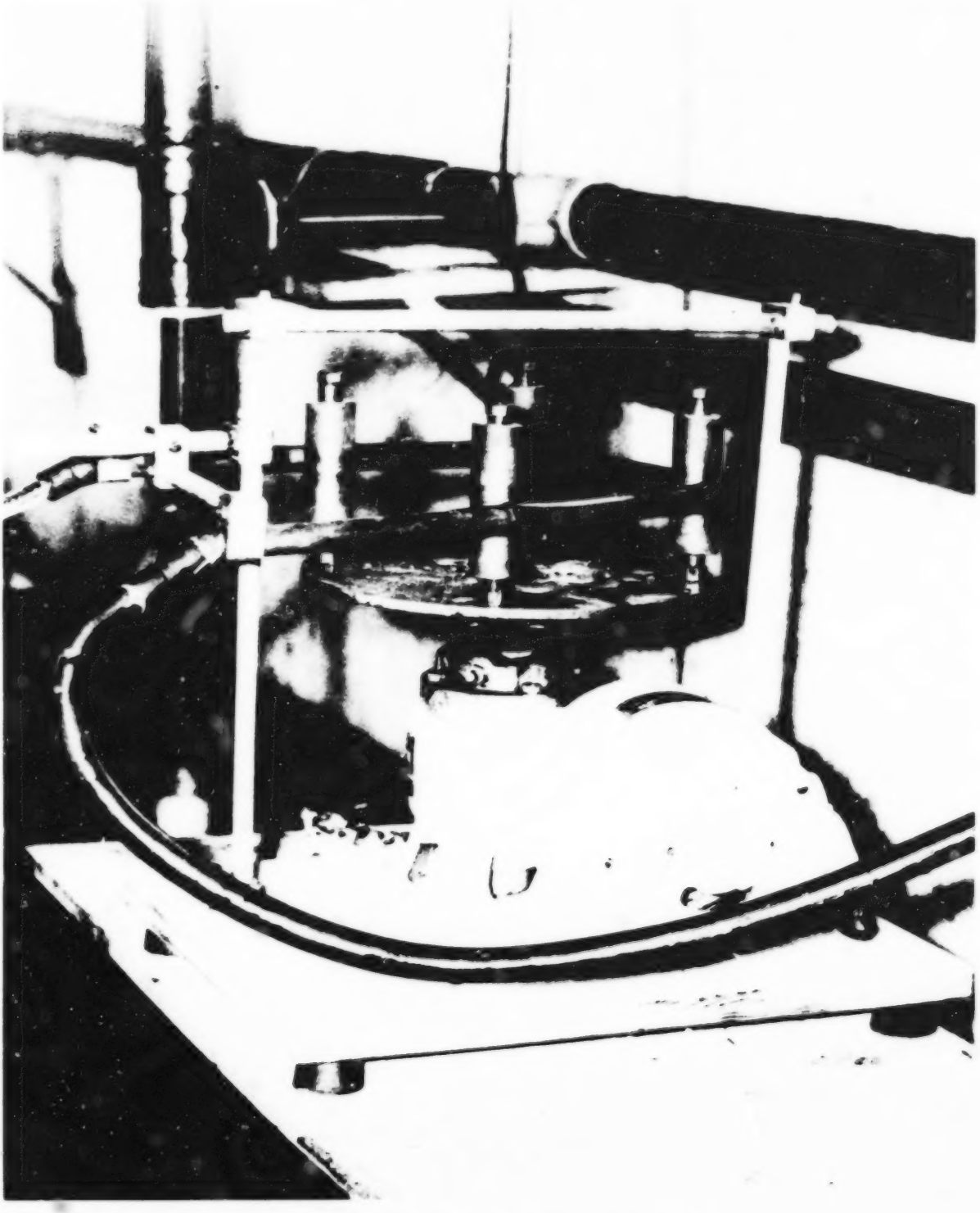

Figure 2. PHOTOGRAPH OF BRITTINGHAM PUMP

chlorinated water, chlorine concentration approximately 200 parts per million, to control slime growths on the shells and interior surfaces of the tanks. The chlorine water was flushed off by hosing and sea water flow resumed.

OPERATION OF EXPERIMENT

Three days prior to the start of the experiment, the ultraviolet lamps were turned on. The daily flushing and chlorination was continued until the beginning of pollution. During the actual experiments, however, chlorination was not practiced; rather, the flats were drained twice daily, the oysters checked for soundness and the flats flushed with the same water supplied to that flat.

A schedule of the operations preliminary to the experiments and during the course of the experiment is shown as follows:

1. Preparation of Flats and Experimental Animals

Transfer approximately 150 oysters to each of the three flats. Adjust water flow to 12 liters per flat per minute. Drain, flush, and chlorinate flats daily. Three days before the start of the experiment turn on ultraviolet lamps. Drain and flush irradiation troughs daily and wipe off ultraviolet lamps to remove accumulated dust and salt spray.

2. Zero Day

Collect composite samples of oysters from flats "A", "B", and "C".

Prepare polluting agent and start flow of pollution to flats "A" and "B".

Collect samples of water from flats "A", "B", and "C" and from discharge of ultraviolet troughs one hour after each reservoir is put into service.

3. First, Second, and Third Days of Experiment

Change pollution reservoirs morning and evening.

Drain and flush with water entering flats morning and evening.

Check oysters for soundness morning and evening.

Sample water and oysters from each of the three flats five times daily.

WATER TREATMENT

Prior to the beginning of the experiments, a program of examination of the sea water supply was undertaken to determine the natural coliform level. It was at first anticipated that the water might be used without treatment, particularly if the coliform MPN's were low and of constant concentration. However, examinations of the water demonstrated that the water was quite variable in coliform content. Approximately 25 percent of the samples examined showed coliform MPN's more than 700 and these high values occurred with wide fluctuations and at irregular intervals. Since we required water of at least constant coliform content we decided that the best way of assuring uniformity was to reduce the coliform content to zero or near zero by some means of sterilization. Chlorination was the obvious first choice. Recognizing, from the work of Galtsoff (9), that residual chlorine has an adverse effect on the activity of oysters, provision was made in the water treatment system for dechlorination with sodium thiosulphate.

Preliminary observations suggested that, even after dechlorination, water so treated interfered with the feeding activity of the oysters. This was evidenced by a reduction in the proportionate numbers of animals showing fecal deposits and the relatively small quantity of deposits. It was, therefore, considered advisable to conduct comparative tests to determine more accurately whether water so treated was, in effect, influencing the feeding activity of the oysters.

The results of these comparative tests are described in Part II of this report. These tests demonstrated that under the conditions of our experiment, the addition of chlorine with or without dechlorination interfered with the feeding activity of the oysters.

It was therefore necessary to develop a substitute method for the removal of the coliforms from the water. The method that appeared to be most promising was the use of ultraviolet light. It was speculated that, if this method were effective, it might have the advantage of producing less extensive changes in chemical

constituents of the sea water to which the oysters are sensitive. This method of water treatment proved to be quite effective in the destruction of the coliform organisms, and at the same time, ultraviolet treated water was demonstrated to have no influence on the feeding activity of oysters. A description of the experiments conducted to evaluate the effectiveness of this method and the influence of ultraviolet treated water on the activity of oysters will be found in Part III.

LABORATORY METHODS

The coliform tests conducted in these experiments were performed by methods essentially as described in "Recommended Procedures for the Bacteriological Examination of Shellfish and Shellfish Waters" (10). The following modifications to the procedure, however, were made.

1. The dilution water was Butterfield's phosphate buffered dilution water as recommended in "Standard Methods for the Examination of Water, Sewage, and Industrial Waste", Tenth Edition (11).

2. Dilution of the oysters after shucking was made by weight rather than by volume as recommended in the 1946 Procedures.

3. Grinding of the diluted sample was done in a Waring Blender. Grinding time was 60 seconds.

The presence of coliform organisms was demonstrated by confirmation of gas positive lactose broth tubes in brilliant green lactose bile broth. The presence of gas in any amount at the end of 24 and 48 hours incubation at 35°C was considered evidence of the presence of coliform organisms.

Part II

THE INFLUENCE OF CHLORINATION AND DECHLORINATION OF SEA WATER ON THE FEEDING ACTIVITY OF OYSTERS

In the preliminary design of the experiments to determine the bacterial relationships between oysters and the overlying water it became necessary to provide a system for the sterilization of the water to reduce the coliform content to a constant density. The method of obvious choice was chlorination. However, recognizing that it has been demonstrated (9) that chlorine even in quantities as small as .05 parts per million interferes with the feeding activity of oysters, provisions were made for the removal of the excess chlorine by sodium thiosulphate. During the course of the evaluation of the experimental equipment, it was observed that oysters receiving chlorinated and dechlorinated sea water showed evidence of reduced activity. To establish that the system of sterilization was responsible, a series of experiments were conducted.

Chlorination of the water was carried out in a tank of approximately 400 liters capacity provided with a series of around the end baffles. With the rate of flow in use, 24 liters per minute, the chlorine contact time was 17 minutes. A sufficient amount of chlorine to produce a

TABLE 2. INFLUENCE OF CHLORINATION AND DECHLORINATION OF SEA WATER ON ACTIVITY OF OYSTERS

Hours of exposure		Percent active oysters: Control flat	Percent active oysters: Test flat	Conditions
		Experiment 1		
8		67	40	Average chlorine residual 0.59 ppm after 15 minutes contact. Water dechlorinated after chlorine contact
16		91	51	
7		88	44	
16		99	56	
8		90	40	
16		90	42	
	Average	88	45	
6		78	74	Chlorination and dechlorination stopped
		Experiment 2		
16		77	48	Average chlorine residual 0.23 ppm after 15 minutes contact. Water dechlorinated after chlorine contact
8		65	33	
16		73	45	
8		80	47	
	Average	74	43	
16		80	77	Chlorination and dechlorination stopped

residual of 0.1 to 0.5 parts per million after contact time was fed to the water by means of a solution feed hypochlorinator. Dechlorination was accomplished by feeding a solution of sodium thiosulphate. The dosing mechanism was a simple adjustable constant drip device which fed the solution from a reservoir maintained at a constant level.

Two oyster flats were set up, each containing 110 experimental animals. One flat was supplied water chlorinated and dechlorinated as outlined above and the other the same water without treatment. Flows to both flats were maintained at equal rates. Operation of the chlorination and dechlorination processes were checked at frequent intervals by orthotolidine test. Residual chlorine was always found in the water after 17 minutes contact but no trace of chlorine was ever detected in the dechlorinated water entering the experimental flat. The activity of the oysters in both the control and test flat was observed after periods of furnishing the flats flowing treated or untreated sea water. Activity was judged by the presence or absence of fecal deposits. At the end of the experiment, the flat receiving chlorinated water was fed raw water and an activity count was taken. Results are shown in Table 2.

In the first two experiments conducted, 45 and 43 percent of the animals subjected to treated water demonstrated activity during observational periods varying from 6 to 16 hours. The controls receiving untreated water showing 88 percent activity in the first experiment and 74 percent activity in the second. It should be noted that when the flats receiving treated water were supplied with untreated water the extent of activity became the same as that in the control flats. Furthermore, in order to eliminate any possibility of variation due to animals, those that received treated water in the first experiment were used as controls in the second. Inhibition of activity was manifested not only by a reduction in the number of animals showing fecal deposits but also in the quantity of fecal deposits discharged. The oyster receiving treated water showed a much lower quantity of fecal discharges during the same experimental period than the animals in the control flats.

Sodium thiosulfate, even in greater concentrations than those used for dechlorination, did not show any inhibitory effect on activity of oysters. This was tested in the experiment summarized in Table 3.

TABLE 3.
INFLUENCE OF SODIUM
THIOSULFATE ON OYSTER ACTIVITY

Hours of exposure	Percent active oysters		
	Control flat	Test flat	
6	79	75	2.5 ppm Sodium Thiosulfate applied to water entering experimental flat
10	79	75	
9	84	86	

Table 4 lists the results of experiments in which aeration and filtration through an activated carbon were used as dechlorinating agents.

In tests II and III of the experiment, aeration was used after chlorination and dechlorination to determine whether the water could be restored to its non-inhibitory condition. Aeration was not effective. Once the water was treated with chlorine, it became inhibitory and neither dechlorination with thiosulfate nor aeration, changed the character of the water. It can also be seen that there was no residual effect on the oysters by the exposure to the inhibitory water. After changing from test to control conditions, activity of the oysters was restored. The effect of chlorination and dechlorination of the water on activity was rapid. This can be seen from the results of the third day of these experiments when the flats were reversed.

TABLE 4. OYSTER ACTIVITY AFTER EXPOSURE TO VARIOUS CONDITIONS

Experimental conditions	Control flat	Test flat	Percent active oysters	
			Control flat	Test flat
I. 24 Hrs. exposure to untreated water.	C	B	84.5	92
7 Hrs. exposure to chlorinated and dechlorinated water.			84	34
16 Hrs. exposure to chlorinated and dechlorinated water.			86	57
II. 8 Hrs. exposure to chlorinated, dechlorinated, and aerated water.	C	B	84	38
24 Hrs. exposure to chlorinated, dechlorinated, and aerated water.			90	46
III. 24 Hrs. exposure to untreated water.	B	C	84	90
6 Hrs. exposure to chlorinated, dechlorinated, and aerated water.			83	26
7-1/2 Hrs. exposure to chlorinated, dechlorinated, and aerated water.			90	38
IV. 19 Hrs. exposure to untreated water.	B	C	87	80
5 Hrs. exposure to chlorinated, dechlorinated water.			90	40
20-1/2 Hrs. exposure to chlorinated, dechlorinated water.			90	49
V. 24 Hrs. exposure to untreated water.	B	A	87	83
5 Hrs. exposure to chlorinated, charcoal filtered water.			90	38
20-1/2 Hrs. exposure to chlorinated, charcoal filtered water.			90	36
VI. 7 Hrs. exposure to charcoal filtered water.	A	C	76	66
24 Hrs. exposure to charcoal filtered water.			82	82
VII. 24 Hrs. exposure to untreated water.	A	B	76	75
7-1/2 Hrs. exposure to chlorinated, dechlorinated and charcoal filtered water.			89	45
13 Hrs. exposure to chlorinated, dechlorinated and charcoal filtered water.			88	46
7-1/2 Hrs. exposure to untreated water.			89	70
VIII. 27-1/2 Hrs. exposure to untreated water.	B	A	85	75
4 Hrs. exposure to charcoal prefiltered, chlorinated and dechlorinated water.			85	51
20 Hrs. exposure to charcoal prefiltered, chlorinated and dechlorinated water.			96	58
28 Hrs. exposure to charcoal prefiltered, chlorinated and dechlorinated water.			92	63
16 Hrs. exposure to charcoal filtered water.			89	70
6 Hrs. exposure to untreated water.			90	83

Ninety percent of the oysters in flat "B" showed activity during the 24 hours when they were used as control animals, and the percent activity reduced to 26 on the subsequent 6 hours when they were used as test animals. Similar behavior can be seen in the results shown in the fourth section of the table.

The last four sections of Table 4 show the results of tests to determine the influence of filtration through activated carbon. In test V, the activated carbon filter alone was used as the dechlorinating agent. Sodium thiosulfate was not added. It can be seen in the fifth section of Table 4 that the same inhibitory effect on activity occurred with the use of the activated carbon filter as with sodium thiosulfate as dechlorinating agents.

Whether the use of an activated carbon filter alone produced an inhibitory water is not clear from the results of test VI. The defference in activity between the test and control flats during the first 7 hours may be suggestive of inhibition, but at least the same percentage activity was noted in both flats after 24 hours.

In tests VII and VIII we passed the chlorinated and dechlorinated sea water through the activated carbon filter to determine if the inhibiting factor could be absorbed or filtered out. There was no change in the relative percentages of active animals in the test and control flats from the results obtained with the use of thiosulfate alone. Again the oysters in the test increased in activity when fed untreated water.

The next series was designed to determine if the factors responsible for inhibiting activity could be removed by filtration through activated carbon. The results in the last section of Table 4 show that this was not accomplished.

DISCUSSION

It could only be concluded from these experiments that, at least with the water we were using and under condition of continuous flow, chlorinated and dechlorinated water had an influence on the feeding activity of the oysters. No further investigation was made to determine the actual factor responsible for this inhibition of activity. The evidence collected in these experiments, however, is strongly suggestive that the action is a subtractive one in which chlorine either changes or destroys certain factors that influence the activity of oysters. This is demonstrated by the fact that once chlorination of the water had been carried on, no treatment for the removal of chlorine was successful in restoring the water to a condition which would promote activity.

It has been reported that minute quantities of substances influence the feeding activity of oysters. Collier et al (7) has noted an association between the presence of dissolved organic substances and increased activity of oysters. We did not determine whether such substances are changed or destroyed by the action of chlorine in the concentrations used for the sterilization of sea water.

It is recognized that chlorinated and dechlorinated sea water has been used for a number of years in England (3) (4) (5) and later in Portugal (6) in the purification of mussels and oysters. However, in all of these installations, the fill and draw method is utilized in which the water is chlorinated for a considerable time in a chlorine contact tank and dechlorinated at the time of transfer to the shellfish purification tank, and the water then held in the purification tank for a period of 12 to 24 hours. In this respect, particularly the quiescent standing in contact with the shellfish, the process differs from the continuous flow procedure we had to use in the pollution studies.

Our results confirm the work of Galtsoff (9) to the extent that we also found inhibition by as little as .05 parts per million of chlorine. Our findings are also similar to his in that the oysters receiving treated water would not always show complete lack of activity, rather in many instances the rate of activity was only markedly reduced. Dr. Galtsoff did not study the influence of dechlorinated water.

The experiments described in this section were aimed primarily at the evaluation of the experimental conditions established for the oyster pollution studies. However, the observed definite inhibition of activity of oysters by sea water treated with chlorine and then dechlorinated would suggest that in shellfish purification processes, particularly of the continuous water flow type, the effect of added chemicals should be thoroughly investigated before their use.

Part III

ULTRAVIOLET RAY TREATMENT OF SEA WATER AND ITS INFLUENCE ON THE FEEDING ACTIVITY OF OYSTERS

In preliminary experiments in studies on the relative coliform content of oysters and the overlying water, it was found that chlorination and dechlorination influenced the feeding activity of oysters. In view of these observations it became necessary to investigate other methods of removal or destruction of coilform organisms in the water. Since the literature mentions a number of chemicals which adversely effect the activity of the oysters it was decided to evaluate the feasibility of the use of ultraviolet ray treatment as a means of sterilization and at the same time to determine whether ultraviolet ray treated water had any influence on the feeding activity of oysters.

DESIGN OF ULTRAVIOLET RAY WATER TREATMENT UNITS

Recent reports (1) (2), have indicated that ultraviolet light, using properly designed equipment, is effective in the destruction of coliform organisms in water. The apparatus used in our studies is a modification of an apparatus suggested by Buttolph (3). The unit consisted of a bottom section, essentially an open trough 8 inches wide, 36 inches long, and 6 inches high with a longitudinal baffle 2-1/2 inches high placed along the center line of the trough. A lamp housing, fitting on the trough is a semicircular arch shaped reflector, lined with specular finish aluminum reflector material. Each reflector was provided with two 30 watt ultraviolet germicidal lamps.

Four such units were connected in series. At the water flow of 40 liters per minute in the experiments, theoretical detention time in the system was 70 seconds. Actual minimum detention time, as determined by dye was 45 seconds.

This design provided an apparatus which could be easily dismantled for cleaning. Experience with similar apparatus has shown that when used with sea water in this area the surfaces quickly are covered with deposits of particulate matter or of slime and other similar materials. Since it has been pointed out by Buttolph (3) that such deposits on the surfaces of the lamps and reflectors interfere seriously with the transmission of ultraviolet energy, we chose an apparatus that could be cleaned at frequent intervals with a minimum of effort. While the units were in use, a daily routine of maintenance was followed. The troughs were drained and flushed and the lamps and reflector units were wiped with a damp cloth to remove accumulated dust and salt water spray. In addition, the output of the lamps was measured periodically with a germicidal watt meter.

INFLUENCE OF ULTRAVIOLET TREATED WATER ON ACTIVITY OF OYSTERS

After preliminary tests to determine optimum operating conditions for coliform reduction, experiments were conducted to determine the effect of ultraviolet treated of sea water on the feeding activity of oysters. The methods were similar to those followed in the experiments with chlorinated and dechlorinated sea water. Oyster flats, each containing 150 oysters were supplied with sea water at a rate of 12 liters or more per minute. The test flat received ultraviolet treated water and the control flat received water from the same source without treatment. Activity counts of oysters were taken at intervals shown in Table 5.

Three sets of observations made on two successive days showed that ultraviolet treatment of water did not influence the feeding activity of the oysters. The percentages of active animals in the control and test flats were not significantly different.

TABLE 5.
EFFECT OF ULTRAVIOLET TREATED WATER ON ACTIVITY OF OYSTERS

Experimental condition	Percent active oysters	
	Control flat	Test flat
24 Hours exposure to untreated water	88.5	89.5
8 Hours exposure to ultraviolet treated water	87.5	89.5
24 Hours exposure to ultraviolet treated water	90	94.5
8 Hours to ultraviolet treated water	89.5	87.5

BACTERICIDAL EFFICIENCY OF UV UNITS

To be assured of the effectiveness of the units to destroy the coliform organisms in the greatest density anticipated in the raw sea water, tests were conducted in which the raw water was seeded with sufficient cotton filtered raw sewage to produce coliform loads up to MPN 7,900 per 100 ml. Appropriate quantities of diluted sewage were introduced continuously into the sea water supply by means of a Brittingham pump. The mixture was then flowed through the ultraviolet treatment units at the same rate of flow that was required in the experiments on the rate of accumulation of coliforms by oysters.

The results of these tests to determine the bactericidal efficiency of the ultraviolet units are shown in Table 6. Twenty-five sets of samples were examined during the course of three experiments. The ultraviolet treatment units consistently produced water showing coliform MPN's less than 1.8 when submitted raw water with coliform MPN's derived mostly from raw sewage, of 79 to 7,900. The bactericidal action was not complete. Growth was observed in many of the presumptive broth tubes, particularly in the 10 ml volumes, but in no instances were coliform organisms recovered.

TABLE 6.
EFFICIENCY OF ULTRAVIOLET TREATMENT OF WATER IN DESTRUCTION OF COLIFORM ORGANISMS

Test Number	Coliform MPN per 100 ml	
	Before Treatment	After Ultraviolet Treatment
1	7,900	<1.8
2	4,900	<1.8
3	2,300	<1.8
4	2,200	<1.8
5	2,300	<1.8
6	2,300	<1.8
7	4,900	<1.8
8	2,300	<1.8
9	1,100	<1.8
10	1,700	<1.8
12	790	<1.8
13	4,900	<1.8
14	79	<1.8
15	1,400	<1.8
16	490	<1.8
17	460	<1.8
18	490	<1.8
19	1,100	<1.8
20	1,300	<1.8
21	3,500	<1.8
22	2,400	<1.8
23	700	<1.8
24	330	<1.8
25	1,300	<1.8

A control flat was included in the experiments described in Parts I and IV of this report. The oysters were treated exactly as those in the two experimental flats except that they were supplied with ultraviolet treated water only. The purpose of the control flat was to detect contaminations or failures in the water treatment system, as well as to furnish a background level of coliform content of water and oysters. Samples of water and oysters were examined exactly the same as the samples taken from the test flats. In addition, the effluent of the ultraviolet treatment unit was sampled periodically. Three 100 ml portions of each treated water sample were planted. A summary of the coliform results obtained during the course of the experiments is presented in Table 7.

TABLE 7. COLIFORM MPN'S OF ULTRAVIOLET TREATED SEA WATER AND OF WATER AND OYSTERS FROM CONTROL FLAT C

	Water, UV Effluent MPN			Water Flat C MPN			Oyster Flat C MPN			
	<0.41	0.41	>0.41	<1.8	2.0-4.5	>7.8	<18	20-45	78-490	>490
No.*	173	2	1	244	8	5	200	45	11	2
%**	98.3	1.2	0.5	95	3.1	1.9	77.5	17.5	4.3	0.8

*Number of samples showing stated coliform MPN's or less
**Percent of total samples of each type examined.

The data are presented in the form of numbers of samples showing stated coliform MPN's and the percentage of total samples of each type falling in the stated MPN group or less. Of the 176 samples of ultraviolet unit effluent collected during a period of nine months, 98.3 percent showed the absence of coliform organisms from three 100 ml portions, equivalent to MPN less than 0.41. One half of one percent showed coliform MPN's more than 0.41. Of the samples examined from control flat "C", 95 percent of the waters and 77.5 percent of the oysters showed indeterminate MPN values at the low level, less than 1.8 for the water and less than 18 for the oyster. Of the samples in which coliforms were found, the majority of both waters and oysters showed no more than two tubes positive in the lowest portions planted. The MPN ranges are shown in Table 7.

DISCUSSION

The experiments described in this section showed that ultraviolet ray treatment was effective in the destruction of coliform organisms in sea water. Sea water, seeded with raw sewage to produce coliform MPN's as high as 7,900 per 100 ml was effectively sterilized by our ultraviolet treatment unit.

The fact that this method of treatment produced water showing the absence of coliform organisms from three 100 ml volumes in 98 percent of 176 samples of sea water further confirms the reliability of this method of sterilization.

In contrast to the results obtained with chlorinated and dechlorinated sea water, ultraviolet treatment of water did not influence the feeding activity of oysters.

The reliability of ultraviolet treatment with waters of low turbidity would suggest the possibilities of its use in treating water for the purification of shellfish, particularly oysters. It is recognized, however, that the penetration of ultraviolet energy is greatly inhibited by the presence of suspended matter and therefore the treatment might be relatively ineffective in highly turbid waters. If ultraviolet treatment were to be used to sterilize water for shellfish purification, for example, clarification of the water, possibly by settling only, would be indicated in certain installations. One could also expect difficulties with recirculated water unless a clarification process was included in the water treatment system. Ultraviolet treatment, however, has the advantage of compactness, simplicity of design, and low cost of operation.

Part IV

THE RATE OF BACTERIAL ACCUMULATION BY THE OYSTER, CRASSOSTREA VIRGINICA, ON THE GULF COAST

Eighteen experiments were conducted throughout more than one calendar year from December 1954 to February 1956. The schedule of conducting these experiments was arranged to attempt to encompass the possible seasonal variations which might have an influence on the rate of bacterial accumulation by oysters. The times of the year at which these experiments were conducted together with a graph of the average daily water temperature during the period covered by these experiments is shown in Figure 3.

The results of each experiment are presented in individual tabulations in the Appendix. However, to illustrate the sampling schedule and the examinations conducted in each experiment, the results obtained in experiment number one are presented in Table 8.

TABLE 8. RUN NO. 1 -
RELATIVE COLIFORM CONTENT OF OYSTERS AND OVERLYING WATER

December 13 to December 16, 1954

Date	Time	Coliform MPN Per 100 ml						Estimated coliforms per 100 ml added to Flat A
		Flat A		Flat B		Flat C		
		Water	Oyster	Water	Oyster	Water	Oyster	
12-13-54	08:00		20		20		20	530
	11:00	230		49		<1.8		
	19:00	330		33		<1.8		710
12-14-54	07:00	490	3300	33	130	<1.8	<18	1000
	10:00	490	2300	350	490	<1.8	20	
	14:00	1100	4900	79	490	<1.8	<18	
	19:00	490	4600	46	700	<1.8	<18	
	22:00	1300	2200	49	490	<1.8	<18	
12-15-54	07:00	3500	4900	49	1100	7.8	<18	1300
	10:00	490	4900	95	1300	<1.8	<18	
	14:00	700	7900	79	790	<1.8	<18	
	19:00	330	7900	33	790	<1.8	<18	
	22:00	700	4600	79	330	<1.8	<18	830
12-16-54	07:00	490	7900	46	700	<1.8	<18	1100
	10:00	1300	7900	110	340	<1.8	<18	
	14:00	460	13,000	130	1300	<1.8	20	
	19:00	490	3300	79	2300	<1.8	<18	
	22:00	490	3100	49	790	<1.8	<18	

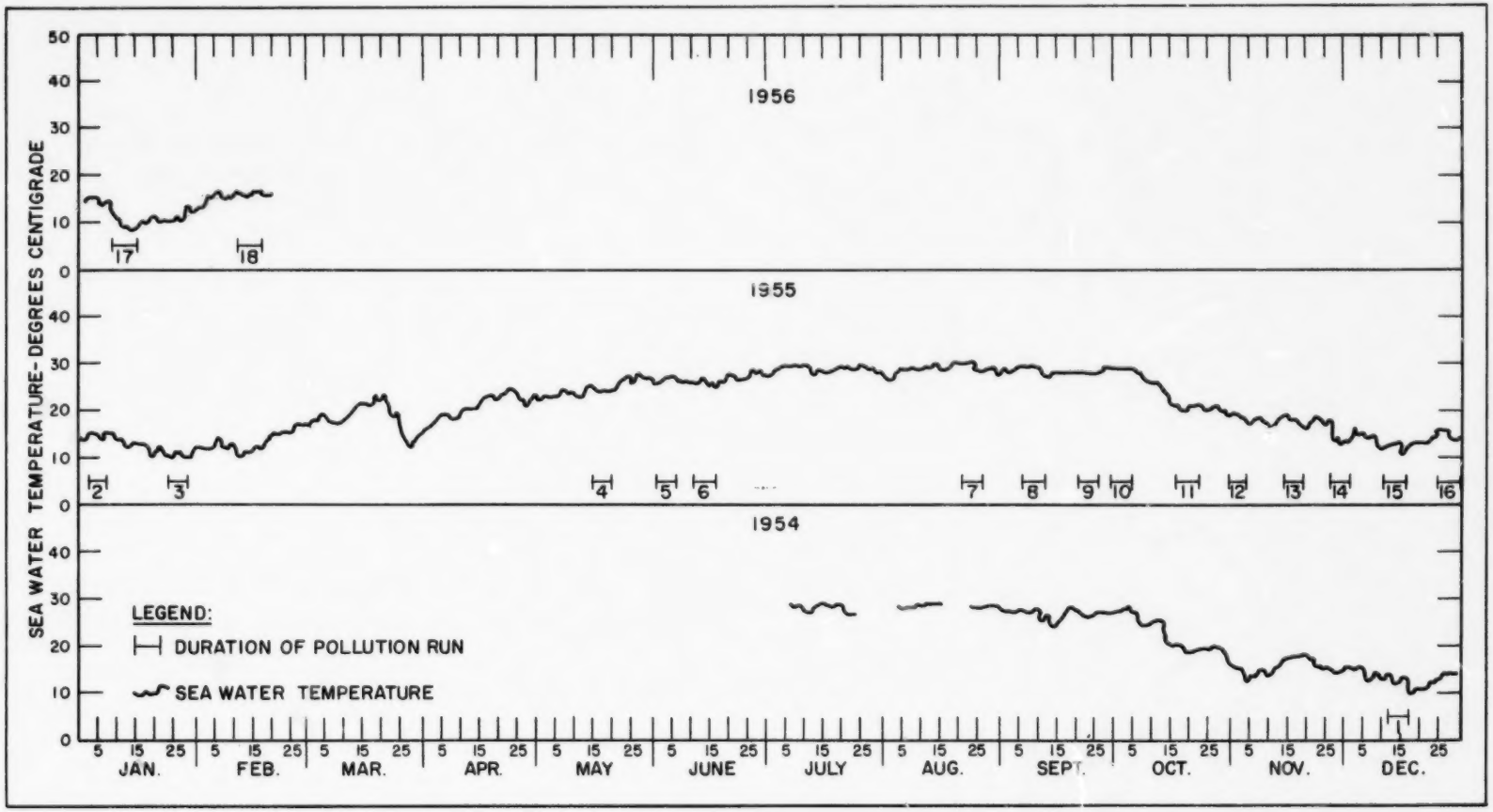

Figure 3. SCHEDULE OF EXPERIMENTS AND RECORD OF SEA WATER TEMPERATURES

This table shows the results of examinations conducted on the first day, which was actually a period to establish bacterial equilibrium between the water and the oyster, and on samples collected during the three days of the actual experiment. The results obtained in Experiment 1 are quite typical of those obtained in all of the experiments.

It can be noted that the oysters in the experimental flats had a coliform MPN of 20 at the start of the experiment and that equilibrium with the increased coliform content of the water was accomplished 24 hours later. Control flat "C" showed the presence of coliform organisms in only one of 17 samples of the water and in two of 17 samples of the oysters. The table is also a typical representation of the variation in coliform MPN of the water and oysters in flats "A" and "B".

The figures in the column "Estimated Coliforms added to flat 'A'" are based on plate counts of the diluted suspensions of coliform organisms prepared for addition to the water in the pollution reservoir.

All of the results were submitted to Dr. E.K. Harris, Chief, Statistical Services, Sanitary Engineering Center, for statistical analysis. A report on the analysis is presented in Part IV. In general, the analysis indicates the following:

Water and oyster MPN's observed in each flat during each run were plotted on separate log-probability grids. The data almost always showed good agreement with a straight line relation, as would be expected from the well-known theory that repeated MPN's obtained from a common source conform to a log-normal distribution. Ratios of geometric mean MPN in oysters to geometric mean MPN in water were therefore used to summarize the experiments.

A study of the relationship between water temperature and these ratios revealed a definite pattern. This can be seen in the data presented in Table 9.

Most outstanding is that all of the oyster-water coliform ratios obtained in runs 11 to 15 inclusive are much higher than in any other experiment. This is true for both flats "A" and

TABLE 9. RELATIONSHIPS OF MEAN OYSTER/WATER COLIFORM RATIOS TO WATER TEMPERATURE AND SEASON

Experiment number	Date	Median water temp. °C	Ratio, $\frac{\text{MPN Oyster}}{\text{MPN Water}}$	
			Flat "A"	Flat "B"
1	12/14/54	17	7.1	8.0
3	1/25/55	16	6.6	8.1
4	5/17/55	24	9.6	2.1
5	5/31/55	26	4.9	3.2
6	6/14/55	26	6.3	6.6
7	8/23/55	30	5.2	8.8
8	9/ 7/55	29	7.9	4.5
9	9/20/55	28	5.3	3.9
10	10/ 4/55	29	4.8	5.2
11	10/18/55	21	26.6	16
12	11/ 1/55	19	27.4	19
13	11/16/55	17	11.5	14
14	11/29/55	14	10.4	13
15	12/14/55	13	13.3	16
16	12/27/55	14	5.6	7.8
17	1/10/56	9	5.8	5.3
18	2/14/56	15	7.2	6.2

"B". The change came quite abruptly and correlates with a sudden change in water temperature. In Figure 3, it can be seen that the median water temperature on October 5 was 29°C. On October 15, ten days later, the water temperature had reduced to 21°C and continued on the downward trend, with a few plateaus and minor rises, throughout all of the time that experiments 11 through 15 were conducted.

It can be seen in Table 9 that the ratios obtained in all of the experiments other than 11 through 15 are quite similar. Since these 5 experiments fell into one water-temperature season, we assembled the other experiments into 3 additional groups, using temperature and season as criteria. These groupings are presented in Table 10.

The ratio of geometric mean oyster coliform MPN to geometric mean water coliform MPN is quite likely the best expression of the average relationship between water and oyster for each experiment. However, variations in activity between individuals exposed to identical environments have been reported many times. Furthermore, changes in activity of oysters with change in water characteristics other than temperature have also been reported. Galtsoff's first studies (1) and (2) on the influence of temperature were reported in terms of percentage of animals active at the various temperatures. Later reports on the influence of environment on pumping rate have also recognized this variation. The latest of these reports is by Loosanoff (3), who records not only the average and maximum pumping rates at various temperatures, but also the total number of oysters treated, the number "open", and the number "pumping". Collier (7) has reported wide variations in pumping rate of the same animal within relatively short periods of time.

To investigate the pattern of variability within each experiment, an "analysis of variance" was performed, using as the basic variable the standard deviation of the log MPN, estimated for each flat from the linear plots on log-probability paper.

Results of this analysis confirmed the effect of water temperature on variability in coliform MPN among oysters. During the relatively extreme temperatures of the December-February and August-September seasons, the variation in MPN among oysters was not significantly different from the variation in water, but during the more moderate temperatures of May-June and October-December increased variation among oysters as compared to water was highly significant.

To illustrate the influence of water temperature on the range of oyster-water coliform ratios, individual ratios within each run were computed by dividing each oyster MPN by the geometric mean water MPN for that experiment*. Then the ratios of all of the experiments in a given water-temperature season group were assembled and from these probability percentiles computed and plotted on log probability paper.

As an example of the pattern of distribution shown by these ratios on a probability plot, the plot for Flat "A" during the August-September season is presented in Figure 4.

*A statistical test described in Part V established that fluctuations in oyster MPN were not correlated with fluctuations in the corresponding water MPN. Thus, in studying oyster-water coliform ratios it was not necessary to relate each oyster MPN to the individual water MPN observed at the same time.

TABLE 10. GROUPING OF OYSTER/WATER RATIOS BY WATER TEMPERATURE AND SEASON

Month	Range of Median Water Temperatures °C.	Experiment No.
December-February	8-16	1, 3, 16, 17, 18
May-June	24-28	4, 5, 6
August-September	27-29	7, 8, 9, 10
October-December	12-21	11, 12, 13, 14, 15

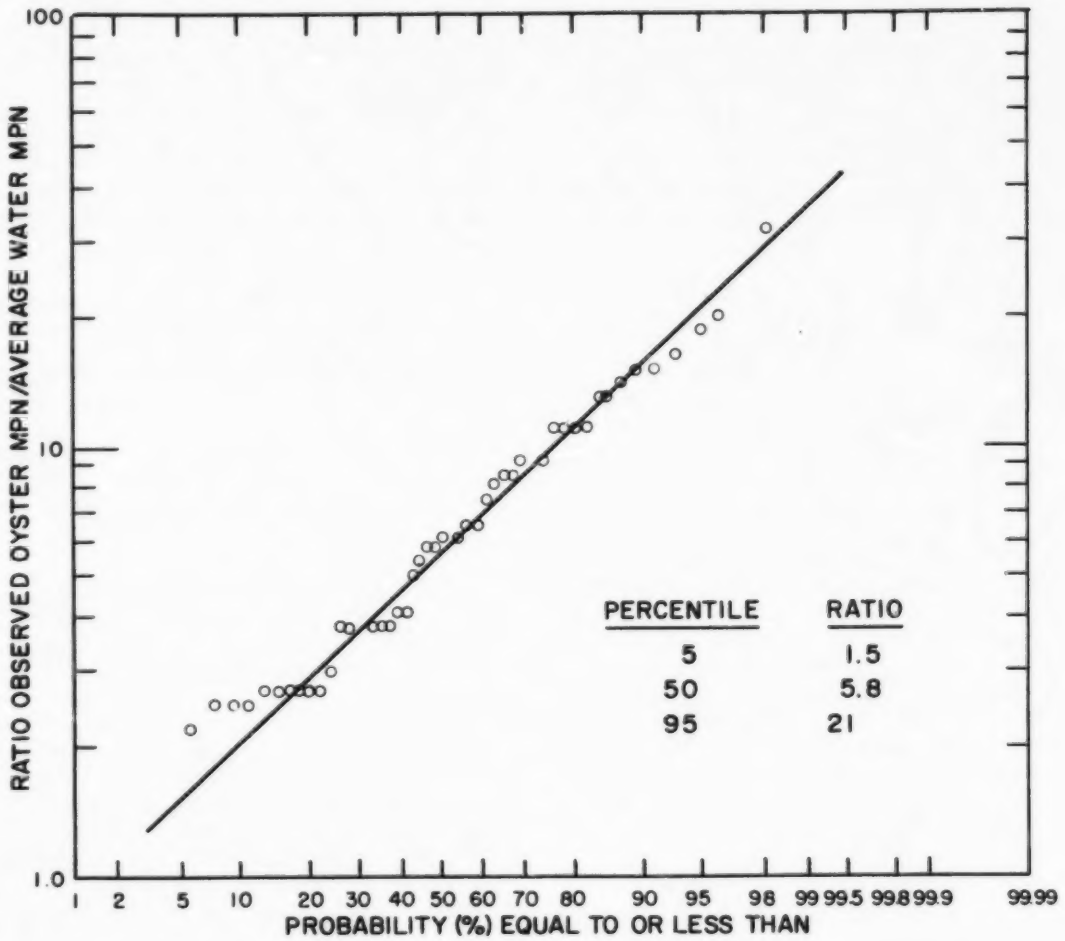

Figure 4. RATIO OBSERVED OYSTER MPN TO AVERAGE WATER MPN

The ratios at the 5, 50 and 95 percentile level for the four water-temperature season groups are presented in Table 11 and Figure 5.

A comparison of the ratios obtained at the various seasons reveals that the season, October-December, is characterized not only by the nighest average ratios but also that the variation in ratios is greater in both flats than at almost any other time. Flat "B" during May and June is the exception.

The lower ratios found in the summer and deep winter could be associated with water temperature. The seasonal changes in ratios correlate quite well with observations of Collier (8), on the pumping rates of a group of oysters throughout the entire year. Collier's studies were conducted at the same location as the one in which our work was done. It is notable that Collier observed increases in the pumping rate when the water started on the downward trend in the fall and a reduction in pumping rate on approach to water temperature below 15 and again in the summer when the water temperature rose to 30°C or above. He also records a period of high pumping rate in the spring when the water temperatures rose to 18 to 20°C. We made no observations during this spring period.

The seasonal changes in rate of bacterial accumulation, particularly the sudden increase in rate of accumulation observed in October also compare well with observations of Butler (12) on the changes in rate of growth of oysters. Our studies and those of Butler's were also conducted at the same location. He found sharp increases in weight and size measurements of oysters in October, and sharp reductions in growth rates in July.

TABLE 11. OYSTER/WATER COLIFORM MPN RATIOS AT FOUR WATER TEMPERATURE GROUPS-5, 50 and 95 PERCENTILE VALUES

Percentile	Ratio - $\frac{\text{MPN oyster}}{\text{MPN water}}$			
	Dec.-Feb. water temp. 8-16°C	May-June water temp. 24-28°C	Aug.-Sept. water temp. 27-29°C	Oct.-Dec. water temp. 12-20°C
		Flat A		
5	1.9	1.8	1.5	2.9
50	6.2	7.4	5.8	16
95	20	31	21	90
		Flat B		
5	2.2	0.7	1.6	3.0
50	7.1	3.8	5.4	15.5
95	23	22	17	82

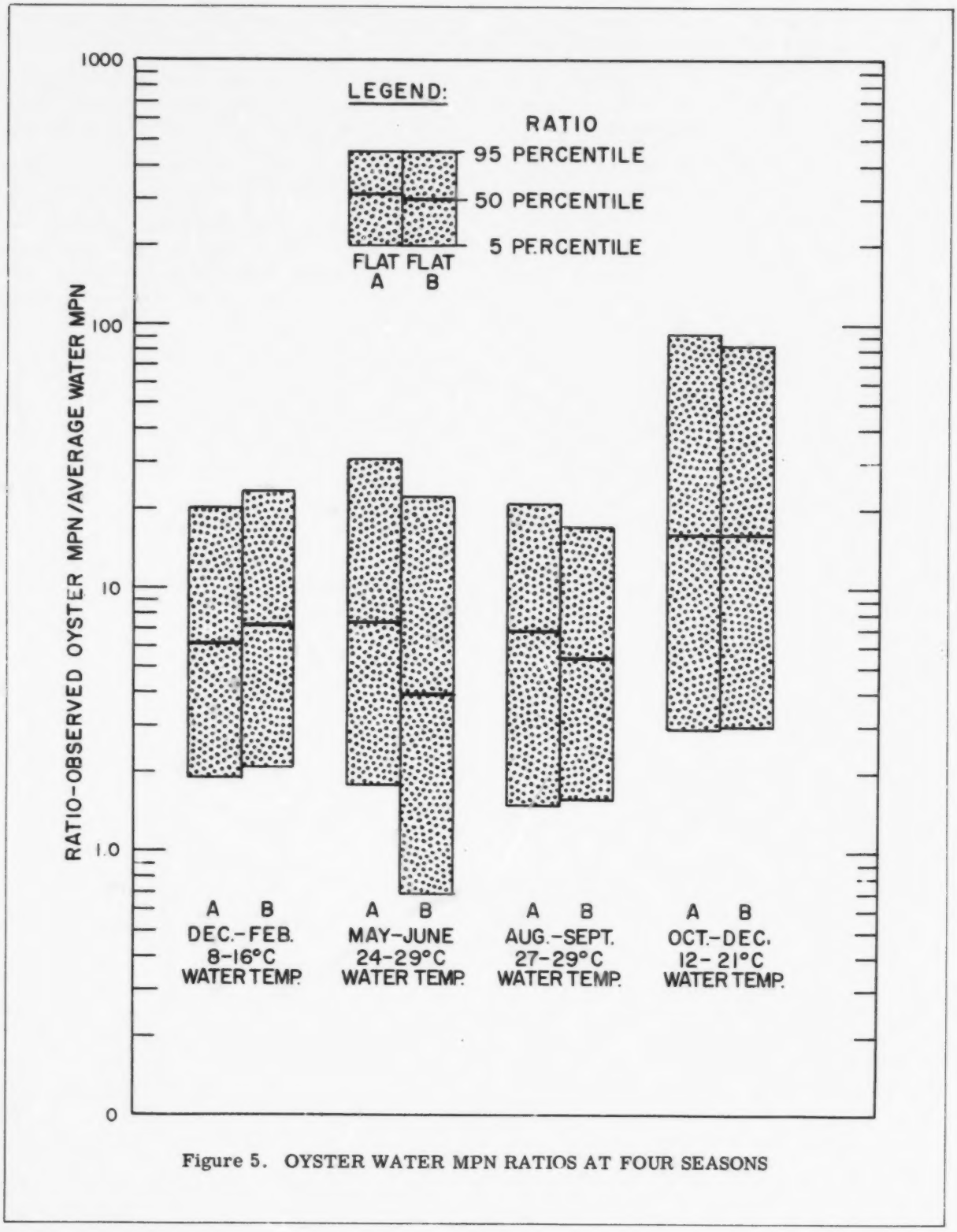

Figure 5. OYSTER WATER MPN RATIOS AT FOUR SEASONS

Part V

STATISTICAL ANALYSIS

E. K. Harris, Ph.D., Chief
Statistical Services

As mentioned in Part IV of this report, water and oyster MPN's observed in each flat during each run were plotted on separate logarithmic probability grids. The data almost always showed good agreement with a straight line relationship as would be expected from well-known theory that replicate MPN's conform to a log normal distribution.

To each plot, the best fitting straight line was drawn by eye and both the mean log (i.e., log of the geometric mean MPN) and the standard deviation of the logs were estimated from this line.

Attention was devoted first to these estimated standard deviations. As expected they showed considerable variation. Our concern was whether this variation was explainable to a significant extent by the different factors involved in the experiments, e.g., oysters versus water, flat (i.e., density of organisms) and season of the year. The statistical technique commonly used in this situation is "analysis of variance". Remember that the variable whose variation is being analyzed is the standard deviation of the log MPN's observed in each flat in water and in oysters during each run.

Since the runs were not equally divided among the four seasons (i.e., temperature ranges), it was most convenient to test for the effects of (1) oysters versus water, and (2) flat within each season. The assignment of runs to the different seasons is given in Table 10 of part IV.

As mentioned in Part IV, results of this analysis confirmed the effect of water temperature on variability in MPN among oysters. During the relatively extreme temperature of the mid-December to February and August to September seasons the variation in MPN's among oysters was not significantly different from the variation in water, but during the more moderate temperatures of May to June and October to mid-December increased variation in oysters as compared to water was highly significant.

In addition, during the December-February and August-September seasons significantly greater variability in both oysters and water appeared in Flat B - which had the smaller coliform density - as compared with Flat A. This difference in variability between the two flats did not occur in the other two seasons.

No significant interactions appeared between the factors, oysters versus water and flat "B" versus flat "A" in the two seasons during which these factors showed significant main effects. In other words, during these seasons oyster MPN's were more variable than water in both flats.

To illustrate the degree of homogeneity attained in the densities of pollution applied, log MPN's in the water of flat "A" over all seasons showed an average standard deviation of .27, practically equal to the theoretically expected value of .26 (based on five-tube, decimal dilution test) under the hypothesis of constant bacterial density over time and throughout the flat. The overall average standard deviation in the water of flat "B" was not much greater, .33, but, as noted above, the difference was found chiefly in waters of moderate temperature where it appeared significant.

Since the log normal distribution of water and oyster MPN's has been confirmed, the ratio of geometric mean oyster MPN to geometric mean water MPN should serve to represent fairly the relationship between pollution in oysters and in the overlying water within a given set of temperature and density conditions, e.g., flat "A", December-February season. The logs of these ratios should be normally distributed

with standard deviation given approximately by the square root of the sum of the variances of mean log oyster MPN and mean log water MPN for that set of conditions. This expected standard deviation assumes that fluctuations in oyster MPN's during any run were not correlated with fluctuations in water MPN's during that run. A strong correlation - presumably in the positive direction - would decrease the variability of oyster/water ratios. As described below, we tested for such correlations and found none.

Table 12 lists for each season and flat the geometric mean (GM) ratio together with observed and expected standard deviations (SD) of the log ratios.

In general, observed and expected standard deviations showed good agreement, from which we may conclude that within each season and flat the observed oyster/water ratios represented random sample values from a homogeneous population. One noticeable exception to this general rule occurred in flat "A" during the October-December runs (runs 11, 12, 13, 14, 15) and was traceable to a sequence of unusually high oyster MPN's in runs 11 and 12.

In fact, it is clear from Table 12 that oyster/water coliform ratios averaged two to three times higher in the fall than in any other season of the year. As may be shown by analysis of variance, during all other months of the year these ratios form a homogeneous group, exhibiting no greater variation that would be expected even under constant temperature and density conditions.

Possible correlations between fluctuations in oyster MPN's and fluctuations in water MPN's within any one run were studied by designating an oyster or water MPN + or -, depending on whether it was above or below the geometric mean MPN for oysters or water, respectively, in that run. If ups and downs in oysters were correlated with similar changes in water, at the same sampling time, then simultaneous water and oyster MPN's should generally both be plus or minus rather than of opposite sign. If no correlation existed, cases of like and unlike signs should occur with equal frequency. Relative frequencies of like and opposite signs were examined in each run with respect to (1) simultaneous water and oyster samples, (2) oyster and the just prior water (usually 3 hours ago), (3) oyster and the second water sample back, and (4) oyster and the third water back - in order to detect any lag effects. In neither flat was any correlation detected, on the average, between fluctuations in oyster MPN's and fluctuations in simultaneous or regressing water MPN's.

This lack of correlation is not surprising. It merely indicates that, because of proper experimental techniques, a stable relationship was established between oysters and water before any sampling was done and that variations in either represented independent random sampling errors plus, the case of oysters, fluctuations in activity level unrelated to any variations in the bacterial density of the water.

TABLE 12. OYSTER/WATER COLIFORM GEOMETRIC MEAN RATIOS AND STANDARD DEVIATIONS OF RATIOS

	December-February		August-September	
	Flat A	Flat B	Flat A	Flat B
G. M. Ratio	5.6	7.4	5.8	5.3
Obs. S.D. of Log Ratio	.15	.10	.10	.18
Exp. S.D. of Log Ratio	.11	.12	.13	.12
	May-June		October-December	
	Flat A	Flat B	Flat A	Flat B
G. M. Ratio	7.0	3.7	16.0	14.3
Obs. S.D. of Log Ratio	.14	.19	.20	.07
Exp. S.D. of Log Ratio	.11	.16	.12	.14

Part VI

DISCUSSION

The experiments in Part IV of this report were undertaken primarily to obtain some fundamental facts on the rate of accumulation of bacteria by the Eastern oyster in the Gulf Coast environment. These data were necessary to furnish information for the establishment of acceptable bacteriological levels for waters in shellfish growing areas and for Gulf Coast oysters as harvested. Controlled laboratory experiments were indicated to eliminate many of the variables in a natural estuary or at least to control some of them. The coliform density of the water was controlled in three levels, one a practical zero, the other two being concentrations ordinarily met with in approved and moderately polluted shellfish growing areas. Eighteen experiments were conducted throughout more than a complete calendar year to determine the influence of temperature and season on the rate of bacteriological accumulation.

The rate and extent of accumulation, within the ranges of bacteriological density of the water in the experiments appeared to be constant at any season. The water-oyster coliform ratios in the two flats which contained measurable amounts of coliform organisms were always similar during the same season. The oyster-water coliform ratios were significantly higher however, during one period of the year than at any other time. This occurred in the late fall following a sharp decline in water-temperature and continued for a period of approximately two months when the water temperatures were above 13^{o} Centigrade. It is significant that the period of highest extent of bacteriological accumulation occurs at a time when probably one of the more intensive commercial harvesting of oysters takes place. This is in distinct contrast to the situation in the middle Atlantic and North Atlantic states, with the reduction of water-temperatures to below 10^{o} Centigrade the oysters activity is lowered sometimes to the point of hibernation during the periods of commercial harvesting. This increased activity during the winter was also observed by other workers who measured activity using other biological methods.

The observations made in parts II and III of this report on the influence of chlorination on the feeding activity and the evaluation of the efficiency of ultraviolet ray treatments of sea water should be of practical significance to those concerned with the design and operation of shellfish purification plants. In the experiments on the influence of chlorination and dechlorination of sea water, it is quite evident that this method of treatment produces a water that interferes with feeding activity of oysters in a flowing-through system of purification, and thus it would be undersirable as a method of sterilization of the sea water. To what extent these results can be transposed to other species is not known; we do know, however, that the Eastern oyster is extremely sensitive to minute quantities of foreign materials in the water. Furthermore, the work of Collier (8) indicates an influence on activity by very small concentrations of arabinose-like substances natural to sea water. The mode of action of chlorination was not investigated, but the experiments seem to indicate that the chlorination either modified or destroyed some component of the water since filtration through activated carbon or aeration was not successful in restoring the water to its original state; thus, materials absorbable by carbon or oxidizable or removable by aeration were not among the factors responsible for the inhibition of activity.

On the other hand, treatment of water with ultraviolet light had no adverse effect. Comparative tests on control and test flats, receiving raw and untreated water, showed the same extent of activity among oysters receiving raw and untreated water. This method of treatment of water was also found to be highly effective in the destruction of coliform organisms. In preliminary experiments it was demonstrated that coliform MPN per 100 ml of sea water could be reduced from as much as 7,900 to less than 1.8 in a simple treatment unit. Evaluation of the treatment unit throughout the sixteen months of experiment also proved its reliability.

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Appendix

TABULATED RESULTS OF INDIVIDUAL EXPERIMENTS

TABLE 1. RELATIVE COLIFORM CONTENT OF OYSTERS AND OVERLYING WATER

Run No. 1 - December 13 to December 16, 1954

Date	Time	Coliform M.P.N. per 100 ml						Estimated coliforms added to flat A (Per 100 ml.)
		Flat A		Flat B		Flat C		
		Water	Oyster	Water	Oyster	Water	Oyster	
12-13-54	8:00 a.m.		20		20		20	530
	11:00 a.m.	230		49		<1.8		
	7:00 p.m.	330		33		<1.8		710
12-14-54	7:00 a.m.	490	3300	33	130	<1.8	<18	1000
	10:00 a.m.	490	2300	350	490	<1.8	20	
	2:00 p.m.	1100	4900	79	490	<1.8	<18	
	7:00 p.m.	490	4600	46	700	<1.8	<18	1000
	10:00 p.m.	1300	2200	49	490	<1.8	<18	
12-15-54	7:00 a.m.	3500	4900	49	1100	7.8	<18	1300
	10:00 a.m.	490	4900	95	1300	<1.8	<18	
	2:00 p.m.	700	7900	79	790	<1.8	<18	
	7:00 p.m.	330	7900	33	790	<1.8	<18	
	10:00 p.m.	700	4600	79	330	<1.8	<18	830
12-16-54	7:00 a.m.	490	7900	46	700	<1.8	<18	1100
	10:00 a.m.	1300	7900	110	340	<1.8	<18	
	2:00 p.m.	460	13000	130	1300	<1.8	20	
	7:00 p.m.	490	3300	79	2300	<1.8	<18	
	10:00 p.m.	490	3100	49	790	<1.8	<18	

TABLE 2. RELATIVE COLIFORM CONTENT OF OYSTERS AND OVERLYING WATER

Run No. 2 - January 3 to January 6, 1955

Date	Time	Coliform M.P.N. per 100 ml						Estimated coliforms added to flat A (Per 100 ml.)
		Flat A		Flat B		Flat C		
		Water	Oyster	Water	Oyster	Water	Oyster	
1-3-55	7:30 a.m.		<18		<18		<18	940
	11:00 a.m.	790		110		<1.8		
	7:00 p.m.	230		130		<1.8		1100
1-4-55	7:00 a.m.	790	1300	46	130	<1.8	<18	730
	10:00 a.m.	790	1300	240	330	<1.8	<18	
	2:00 p.m.	790	1300	170	170	<1.8	<18	
	7:00 p.m.	1300	790	33	490	<1.8	45	720
	10:00 p.m.	1300	4600	33	330	<1.8	<18	
1-5-55	7:00 a.m.	330	1700	33	130	<1.8	<18	990
	10:00 a.m.	460	700	49	460	<1.8	<18	
	2:00 p.m.	490	2800	130	3300	<1.8	330	
	7:00 p.m.	460	3300	79	790	<1.8	230	820
	10:00 p.m.	490	4900	79	460	<1.8	1300	
1-6-55	7:00 a.m.	490	3300	49	430	<1.8	1300	850
	10:00 a.m.	1300	1300	170	330	<1.8	93	
	2:00 p.m.	460	2200	33	330	<1.8	490	
	7:00 p.m.	1100	1100	33	2300	<1.8	3500	
	10:00 p.m.	790	790	33	3300	2.0	330	

Water temperature 13 to 16°C.

TABLE 3. RELATIVE COLIFORM CONTENT OF OYSTERS AND OVERLYING WATER

Run No. 3 - January 24 to January 27, 1955

Date	Time	Coliform M.P.N. per 100 ml						Estimated coliforms added to flat A (Per 100 ml.)
		Flat A		Flat B		Flat C		
		Water	Oyster	Water	Oyster	Water	Oyster	
1-24-55	8:00 a.m.		<18		<18		<18	
	10:30 a.m.	790		49		<1.8		850
	7:00 p.m.	790		170		<1.8		750
1-25-55	7:00 a.m.	330	4900	33	790	<1.8	<18	810
	10:00 a.m.	790	3300	33	490	<1.8	<18	
	2:00 p.m.	1300	4900	79	330	<1.8	<18	
	7:00 p.m.	1300	3300	49	230	<1.8	<18	840
	10:00 p.m.	1300	3300	49	1400	<1.8	<18	
1-26-55	7:00 a.m.	1400	4900	33	790	<1.8	<18	850
	10:00 a.m.	1300	4900	240	790	<1.8	<18	
	2:00 p.m.	490	17000	130	790	<1.8	<18	
	7:00 p.m.	330	7900	110	330	<1.8	<18	1100
	10:00 p.m.	790	7900	170	1300	<1.8	<18	
1-27-55	7:00 a.m.	490	17000	49	1300	<1.8	<18	1100
	10:00 a.m.	1300	7900	130	1700	<1.8	<18	
	2:00 p.m.	330	1800	240	490	<1.8	<18	
	7:00 p.m.	790	4900	130	330	<1.8	<18	
	10:00 p.m.	1100	1700	49	130	<1.8	<18	

Water temperature 9 to 11.5°C.

TABLE 4. RELATIVE COLIFORM CONTENT OF OYSTERS AND OVERLYING WATER

Run No. 4 - May 15 to May 19, 1955

Date	Time	Coliform M.P.N. per 100 ml							Estimated coliforms added to flat A (Per 100 ml.)
		U.V. Eff.	Flat A		Flat B		Flat C		
			Water	Oyster	Water	Oyster	Water	Oyster	
5-15-55						<18		<18	
5-16-55	7:15 a.m.					<18		<18	950
	11:00 a.m.	<0.41	330		110		<1.8		
	2:00 p.m.	<0.41							
	6:00 p.m.	<0.41	460		140		<1.8		950
5-17-55	7:00 a.m.	<0.41	700	2200	130	78	<1.8	<18	900
	10:00 a.m.		640	4900	130	490	<1.8	20	
	2:00 p.m.	<0.41	790	13000	130	1100	<1.8	<18	
	6:00 p.m.	<0.41	330	17000	79	410	<1.8	<18	1030
	9:00 p.m.		790	4900	170	100	<1.8	<18	
5-18-55	7:00 a.m.	<0.41	330	11000	79	1300	<1.8	45	890
	10:00 a.m.		1300	3300	110	170	2.0	130	
	2:00 p.m.	<0.41	700	11000	33	68	2.0	18	
	6:00 p.m.	<0.41	230	7900	49	5400	2.0	45	1300
	9:00 p.m.		330	4900	33	330	<1.8	<18	
5-19-55	7:00 a.m.	<0.41	790	490	49	68	<1.8	20	780
	10:00 a.m.		490	1700	130	92	<1.8	<18	
	2:00 p.m.	<0.41	490	4900	70	140	<1.8	<18	
	6:00 p.m.	<0.41	790	13000	240	18	<1.8	<18	
	9:00 p.m.		490	7000	70	40	<1.8	<18	

TABLE 5. RELATIVE COLIFORM CONTENT OF OYSTERS AND OVERLYING WATER

Run No. 5 - May 26 to June 2, 1955

Date	Time	Coliform M.P.N. per 100 ml							Estimated coliforms added to flat A (Per 100 ml.)
		U.V. Eff.	Flat A		Flat B		Flat C		
			Water	Oyster	Water	Oyster	Water	Oyster	
5-26-55	2:00 p.m.			<18		<18		<18	
5-29-55	7:00 a.m.					<18		<18	
5-30-55	7:00 a.m.			<18		<18		18	810
	11:00 a.m.	<0.41	490		49		<1.8		
	6:00 p.m.	<0.41	490		170		<1.8		1300
5-31-55	7:00 a.m.	<0.41	350	1400	140	790	<1.8	<18	1100
	10:00 a.m.		700	3100	540	790	<1.8	45	
	2:00 p.m.	<0.41	2400	3300	110	1700	<1.8	20	
	6:00 p.m.	<0.41	790	24000	350	1300	<1.8	<18	1300
	9:00 p.m.		1300	4900	170	700	<1.8	20	
6-1-55	7:00 a.m.	<0.41	1700	2300	140	330	<1.8	<18	1100
	10:00 a.m.		490	4900	79	490	<1.8	<18	
	2:00 p.m.	<0.41	700	7900	79	170	<1.8	<18	
	6:00 p.m.	<0.41	640	2300	240	700	<1.8	45	1100
	9:00 p.m.		1300	4900	240	2400	<1.8	<18	
6-2-55	7:00 a.m.	<0.41	1700	2800	49	130	<1.8	230	1000
	10:00 a.m.		2400	3300	170	490	<1.8	<18	
	2:00 p.m.	<0.41	700	7900	350	330	<1.8	<18	
	6:00 p.m.	<0.41	1100	7900	33	270	<1.8	<18	
	9:00 p.m.		490	7900	49	700	<1.8	<18	

TABLE 6. RELATIVE COLIFORM CONTENT OF OYSTERS AND OVERLYING WATER

Run No. 6 - June 12 to June 16, 1955

Date	Time	U.V. Eff.	Coliform M.P.N. per 100 ml						Estimated coliforms added to flat A (Per 100 ml.)
			Flat A		Flat B		Flat C		
			Water	Oyster	Water	Oyster	Water	Oyster	
6-12-55	7:30 a.m.			20		20		20	
6-13-55	7:30 a.m.			<18		<18		<18	
	11:00 a.m.	<0.41	1300		540		<1.8		890
	2:00 p.m.	<0.41							
	6:00 p.m.	<0.41	1300		350		<1.8		1400
6-14-55	7:00 a.m.	<0.41	790	4900	49	790	<1.8	<18	920
	10:00 a.m.		1700	4900	170	790	<1.8	<18	
	2:00 p.m.	<0.41	1700	11000	79	1100	<1.8	<18	
	6:00 p.m.	<0.41	790	17000	110	1300	<1.8	<18	1000
	9:00 p.m.		1300	4900	350	1300	<1.8	<18	
6-15-55	7:00 a.m.	<0.41	790	2300	46	330	<1.8	<18	1200
	10:00 a.m.		790	2200	130	1100	<1.8	130	
	2:00 p.m.	<0.41	790	13000	110	1100	<1.8	<18	
	6:00 p.m.	<0.41	1700	4900	79	330	<1.8	<18	
	9:00 p.m.		1300	7900	220	490	<1.8	<18	1200
6-16-55	7:00 a.m.	<0.41	1300	3100	130	330	<1.8	<18	1600
	10:00 a.m.		1700	17000	350	1100	<1.8	<18	
	2:00 p.m.	<0.41	950	9500	95	1700	<1.8	<18	
	6:00 p.m.	<0.41	1100	7000	130	2200	<1.8	<18	
	9:00 p.m.		790	22000	140	490	<1.8	<18	

TABLE 7. RELATIVE COLIFORM CONTENT OF OYSTERS AND OVERLYING WATER

Run No. 7 - August 21 to August 24, 1955

Date	Time	U.V. Eff.	Coliform M.P.N. per 100 ml						Estimated coliforms added to flat A (Per 100 ml.)
			Flat A		Flat B		Flat C		
			Water	Oyster	Water	Oyster	Water	Oyster	
8-21-55	7:40 a.m.			170		170		170	
8-22-55	9:00 a.m.			20		20		20	1700
	11:00 a.m.	<0.41	2400		79		<1.8		
	2:00 p.m.	<0.41						<18	
	3:30 p.m.								
	6:00 p.m.	<0.41	950		70		<1.8		1300
8-23-55	7:00 a.m.	<0.41	230	1300	70	790	<1.8	45	1400
	10:00 a.m.		330	4900	79	2400	<1.8	<18	
	2:00 p.m.	<0.41	230	330	33	330	<1.8	2400	
	6:00 p.m.	1+	110	950	17	490	2.0	330	1600
	9:00 p.m.		2200	14000	350	1700	<1.8	<18	
8-24-55	7:00 a.m.	3+	490	2200	170	790	<1.8	16000	1100
	10:00 a.m.		330	4900	70	790	<1.8	20	
	2:00 p.m.	<0.41	490	3300	170	330	<1.8	45	
	6:00 p.m.	<0.41	490	4900	350	2200	<1.8	45	1200

TABLE 8. RELATIVE COLIFORM CONTENT OF OYSTERS AND OVERLYING WATER

Run No. 8 - September 5 to September 9, 1955

Date	Time	Coliform M.P.N. per 100 ml							Estimated coliforms added to flat A (Per 100 ml.)
		U.V. Eff.	Flat A		Flat B		Flat C		
			Water	Oyster	Water	Oyster	Water	Oyster	
9-5-55	8:30 a.m.			40		40		40	
9-6-55	9:00 a.m.			<18		<18		<18	
	10:30 a.m.	<0.41	2400		170		<1.8		1300
	2:30 p.m.	<0.41							
	6:00 p.m.	<0.41	330		130		<1.8		1700
9-7-55	7:00 a.m.	<0.41	790	3300	110	640	920	240	1600
	10:00 a.m.		1300	13000	230	490	<1.8	45	
	2:00 p.m.	<0.41	490	7900	70	790	<1.8	<18	
	6:00 p.m.	<0.41	1300	13000	95	1300	7.8	45	1800
	9:00 p.m.		790	7900	130	490	<1.8	490	
9-8-55	7:00 a.m.	<0.41	460	2300	170	230	<1.8	20	1600
	10:00 a.m.		790	7900	110	220	<1.8	18	
	2:00 p.m.	<0.41	1300	11000	130	490	<1.8	18	
	6:00 p.m.	<0.41	490	3300	70	490	<1.8	<18	1800
	9:00 p.m.		790	3300	79	330	<1.8	<18	
9-9-55	7:00 a.m.	<0.41	1700	9500	110	330	4.5	37	1400
	10:00 a.m.		1100	11000	130	790	<1.8	<18	
	2:00 p.m.	<0.41	2400	7900	79	490	<1.8	40	
	6:00 p.m.	<0.41	790	7000	130	490	<1.8	18	
	9:00 p.m.		490	14000	79	330	<1.8	<18	

TABLE 9. RELATIVE COLIFORM CONTENT OF OYSTERS AND OVERLYING WATER

Run No. 9 - September 18 to September 23, 1955

Date	Time	Coliform M.P.N. per 100 ml							Estimated coliforms added to flat A (Per 100 ml.)
		U.V. Eff.	Flat A		Flat B		Flat C		
			Water	Oyster	Water	Oyster	Water	Oyster	
9-18-55	1:00 p.m.			<18		<18		<18	
9-19-55	8:30 a.m.			78		78		78	
	10:40 a.m.	<0.41	790		170		<1.8		1400
	2:00 p.m.	<0.41							
	6:00 p.m.	<0.41	330		79		<1.8		1600
9-20-55	7:00 a.m.	<0.41	1700	7900	49	1100	<1.8	330	910
	6:00 p.m.	<0.41	1300	2300	49	490	<1.8	<18	1200
9-21-55	7:00 a.m.	<0.41	1300	490	110	330	<1.8	<18	1000
	10:00 a.m.		1300	11000	110	1100	<1.8	<18	
	2:00 p.m.	<0.41	1100	24000	110	330	<1.8	<18	
	6:00 p.m.	<0.41	1100	7900	170	700	<1.8	<18	1700
	9:00 p.m.		790	7900	240	1100	<1.8	20	
9-22-55	7:00 a.m.	<0.41	1700	11000	79	330	<1.8	<18	1100
	10:00 a.m.		1100	4900	130	330	<1.8	130	
	2:00 p.m.	<0.41	1300	3300	79	170	7.8	170	
	6:00 p.m.	<0.41	2200	7900	33	490	7.8	130	1400
	9:00 p.m.		790	3300	79	490	2.0	45	
9-23-55	7:00 a.m.	<0.41	1300	3300	350	9200	<1.8	20	1600
	10:00 a.m.		3500	4900	110	790	<1.8	20	
	2:00 p.m.	<0.41	1100	7000	170	230	<1.8	<18	
	6:00 p.m.	<0.41	1300	4900	110	490	<1.8	<18	

TABLE 10. RELATIVE COLIFORM CONTENT OF OYSTERS AND OVERLYING WATER

Run No. 10 - October 2 to October 6, 1955

Date	Time	U.V. Eff.	Coliform M.P.N. per 100 ml						Estimated coliforms added to flat A (Per 100 ml.)
			Flat A		Flat B		Flat C		
			Water	Oyster	Water	Oyster	Water	Oyster	
10-2-55	7:00 a.m.			<18		<18		<18	
10-3-55	7:30 a.m.			<18		<18		<18	
	10:30 a.m.	<0.41	790		540		<1.8		1300
	2:00 p.m.	<0.41							
	6:00 p.m.	<0.41	1300		130		<1.8		1200
10-4-55	7:00 a.m.	<0.41	490	4900	49	220	<1.8	<18	1500
	10:00 a.m.		5400	3300	79	490	<1.8	<18	
	2:00 p.m.	<0.41	1700	7900	130	490	<1.8	<18	
	6:00 p.m.	<0.41	2400	7000	110	790	<1.8	<18	1800
	9:00 p.m.		790	17000	79	1100	<1.8	<18	
10-5-55	7:00 a.m.	<0.41	2400	3300	130	490	<1.8	<18	1000
	10:00 a.m.		790	7900	79	330	<1.8	<18	
	2:00 p.m.	<0.41	790	4600	79	68	<1.8	<18	
	6:00 p.m.	<0.41	1300	7900	130	790	<1.8	<18	1000
	9:00 p.m.		490	3300	130	490	<1.8	<18	
10-6-55	7:00 a.m.	<0.41	950	7000	79	230	<1.8	<18	1600
	10:00 a.m.		1100	24000	130	790	<1.8	<18	
	2:00 p.m.	<0.41	700	3300	70	790	<1.8	<18	
	6:00 p.m.	<0.41	5400	3300	49	790	<1.8	<18	
	9:00 p.m.		790	4900	170	490	<1.8	20	

TABLE 11. RELATIVE COLIFORM CONTENT OF OYSTERS AND OVERLYING WATER

Run No. 11 - October 16 to October 21, 1955

Date	Time	U.V. Eff.	Coliform M.P.N. per 100 m.						Estimated coliforms added to flat A (Per 100 ml.)
			Flat A		Flat B		Flat C		
			Water	Oyster	Water	Oyster	Water	Oyster	
10-16-55	7:00 a.m.			<18		<18		<18	
10-17-55	8:45 a.m.			20		20		20	
	11:15 a.m.	<0.41	1300		350		<1.8		1100
	2:00 p.m.	<0.41							
	6:00 p.m.	<0.41	460		240		<1.8	20	1300
10-18-55	7:00 a.m.	<0.41	490	35000	110	3500	<1.8	<18	1200
	10:00 a.m.		1300	54000	95	2200	<1.8	<18	
	2:00 p.m.	<0.41	790	24000	240	790	<1.8	<18	
	6:00 p.m.	<0.41	950	13000	110	1100	<1.8	<18	1300
	9:00 p.m.		790	54000	130	3500	<1.8	<18	
10-19-55	7:00 a.m.	<0.41	1100	14000	79	790	<1.8	20	1100
	10:00 a.m.		1300	7900	49	790	<1.8	78	
	2:00 p.m.	<0.41	490	11000	79	1700	<1.8	20	
	6:00 p.m.	<0.41	790	35000	110	3500	<1.8	45	1100
	9:00 p.m.		790	17000	130	5400	<1.8	20	
10-20-55	7:00 a.m.								690
	9:00 p.m.	<0.41	790	54000	49	3500	<1.8	<18	850
10-21-55	7:00 a.m.	<0.41	490	7000	110	490	<1.8	20	980
	10:00 a.m.		790	22000	110	790	<1.8	<18	
	2:00 p.m.	<0.41	490	11000	33	790	<1.8	<18	
	6:00 p.m.	<0.41	490	11000	49	330	<1.8	<18	

TABLE 12. RELATIVE COLIFORM CONTENT OF OYSTERS AND OVERLYING WATER

Run No. 12 - October 30 to November 3, 1955

Date	Time	Coliform M.P.N. per 100 ml							Estimated coliforms added to flat A (Per 100 ml.)
		U.V. Eff.	Flat A		Flat B		Flat C		
			Water	Oyster	Water	Oyster	Water	Oyster	
10-30-55	8:40 a.m.			<18		<18		<18	
10-31-55	8:30 a.m.			<18		<18		<18	
	11:00 a.m.	<0.41	1300		920		<1.8		1100
	2:00 p.m.	<0.41							
	6:00 p.m.	<0.41	1400		540		<1.8		
11-1-55	7:00 a.m.	<0.41	1700	17000	79	3500	<1.8	<18	930
	10:00 a.m.		490	92000	240	5400	<1.8	20	
	2:00 p.m.	<0.41	2400	35000	140	3500	<1.8	<18	
	6:00 p.m.	<0.41	790	54000	170	3500	<1.8	<18	920
	9:00 p.m.		330	92000	240	2200	<1.8	<18	
11-2-55	7:00 a.m.	<0.41	1100	7900	79	2400	<1.8	<18	1100
	10:00 a.m.		1700	54000	170	1700	<1.8	<18	
	2:00 p.m.	<0.41	700	92000	350	2800	<1.8	<18	
	6:00 p.m.	<0.41	240	35000	79	3500	<1.8	<18	990
	9:00 p.m.		490	160000	49	1700	<1.8	<18	
11-3-55	7:00 a.m.	<0.41	490	7000	46	1100	<1.8	<18	940
	10:00 a.m.		1300	17000	140	790	<1.8	<18	
	2:00 p.m.	<0.41	1400	7900	70	950	<1.8	<18	
	6:00 p.m.	<0.41	950	3300	70	2200	<1.8	<18	
	9:00 p.m.		700	11000	33	490	<1.8	<18	

TABLE 13. RELATIVE COLIFORM CONTENT OF OYSTERS AND OVERLYING WATER

Run No. 13 - November 14 to November 18, 1955

Date	Time	U.V. Eff.	Coliform M.P.N. per 100 ml						Estimated coliforms added to flat A (Per 100 ml.)
			Flat A		Flat B		Flat C		
			Water	Oyster	Water	Oyster	Water	Oyster	
11-14-55	8:00 a.m.			18		18		18	
11-15-55	8:00 a.m.			20		20		20	
	10:30 a.m.	<0.41	1100		130		<1.8		1100
	2:00 p.m.	<0.41							
	6:00 p.m.	<0.41	2400		170		<1.8		1300
11-16-55	7:00 a.m.	<0.41	3500	3300	33	490	<1.8	45	1200
	10:00 a.m.		1100	7900	49	2400	<1.8	<18	
	2:00 p.m.	<0.41	1300	7900	240	490	<1.8	<18	
	6:00 p.m.	<0.41	490	35000	70	790	<1.8	<18	1400
	9:00 p.m.		1300	35000	49	9200	<1.8	<18	
11-17-55	7:00 a.m.	<0.41	1700	4900	49	230	<1.8	<18	1300
	10:00 a.m.		2400	7900	110	2200	<1.8	45	
	2:00 p.m.	<0.41	1700	35000	790	5400	<1.8	<18	
	6:00 p.m.	<0.41	490	35000	95	2200	<1.8	<18	1500
	9:00 p.m.		1100	13000	130	1300	<1.8	<18	
11-18-55	7:00 a.m.	<0.41	1300	4900	140	790	<1.8	20	1100
	10:00 a.m.		1300	17000	70	790	<1.8	<18	
	2:00 p.m.	<0.41	1700	24000	49	1300	<1.8	<18	
	6:00 p.m.	<0.41	1300	24000	70	460	<1.8	<18	
	9:00 p.m.		490	17000	350	700	<1.8	<18	

TABLE 14. RELATIVE COLIFORM CONTENT OF OYSTERS AND OVERLYING WATER

Run No. 14 - November 27 to December 2, 1955

Date	Time	U.V. Eff.	Coliform M.P.N. per 100 ml						Estimated coliforms added to flat A (Per 100 ml.)
			Flat A		Flat B		Flat C		
			Water	Oyster	Water	Oyster	Water	Oyster	
11-27-55	7:30 a.m.			20		20			
11-28-55	8:00 a.m.			20		20			
	10:45 a.m.	<0.41	3500		130		<1.8		1100
	2:00 p.m.	<0.41							
	6:00 p.m.	<0.41	790		49		<1.8		1700
11-29-55	7:00 a.m.	<0.41	1300	17000	70	3500	<1.8	<18	1400
	10:00 a.m.		1700	4900	49	1400	<1.8	<18	
	2:00 p.m.	<0.41	1300	11000	70	1700	<1.8	<18	
	6:00 p.m.	<0.41	1300	54000	49	490	<1.8	<18	1400
	10:00 p.m.		2400	17000	33	5400	<1.8	<18	
11-30-55	2:00 p.m.	<0.41	3500	7900	33	790	<1.8	<18	1100
	6:00 p.m.	<0.41	490	35000	49	490	<1.8	<18	1600
	10:00 p.m.		2400	17000	180	270	<1.8	<18	
12-1-55	7:00 a.m.	<0.41	790	24000	79	2400	<1.8	<18	1400
	10:00 a.m.		1300	17000	170	950	<1.8	<18	
	2:00 p.m.	<0.41	1300	11000	49	2200	<1.8	<18	
	6:00 p.m.	<0.41	1100	7900	31	330	<1.8	<18	1400
	9:00 p.m.		790	24000	79	1100	<1.8	<18	
12-2-55	7:00 a.m.	<0.41	1700	13000	49	700	<1.8	<18	1200
	10:00 a.m.		2400	11000	110	700	<1.8	<18	
	2:00 p.m.	<0.41	3500	17000	27	2400	<1.8	<18	

TABLE 15. RELATIVE COLIFORM CONTENT OF OYSTERS AND OVERLYING WATER

Run No. 15 - December 12 to December 16, 1955

Date	Time	U.V. Eff.	Coliform M.P.N. per 100 ml						Estimated coliforms added to flat A (Per 100 ml.)
			Flat A		Flat B		Flat C		
			Water	Oyster	Water	Oyster	Water	Oyster	
12-11-55	7:00 a.m.			<18		<18		<18	
12-12-55	10:00 a.m.			45		45		45	1100
	1:00 p.m.	<0.41	1400		79		<1.8		
	6:00 p.m.	<0.41	790		110		<1.8		1100
12-13-55	7:00 a.m.	<0.41	1100	17000	33	1100	<1.8	<18	1100
	10:00 a.m.	<	790	35000	70	5400	<1.8	45	
	2:00 p.m	<0.41	790	7900	23	1400	<1.8	130	
	6:00 p.m.	<0.41	790	35000	95	1400	<1.8	18	1200
	9:00 p.m.		2200	22000	79	310	<1.8	<18	
12-14-55	7:00 a.m.	<0.41	700	24000	79	3500	<1.8	<18	800
	10:00 a.m.		790	54000	49	490	<1.8	<18	
	2:00 p.m.	<0.41	490	13000	33	2400	<1.8	20	
	6:00 p.m.	<0.41	3500	7900	130	3500	<1.8	<18	900
	9:00 p.m.		490	11000	70	330	<1.8	20	
12-15-55	7:00 a.m.								800
	6:00 p.m.	<0.41	790	7900	49	490	<1.8	<18	900
	9:00 p.m.		1100	13000	33	790	<1.8	<18	
12-16-55	7:00 a.m.	<0.41	2200	3300	33	270	<1.8	45	900
	10:00 a.m.		700	4900	79	330	<1.8	<18	
	2:00 p.m.	<0.41	1700	13000	130	460	<1.8	<18	

TABLE 16. RELATIVE COLIFORM CONTENT OF OYSTERS AND OVERLYING WATER

Run No. 16 - December 25 to December 29, 1955

Date	Time	U.V. Eff.	Coliform M.P.N. per 100 ml						Estimated coliforms added to flat A (Per 100 ml.)
			Flat A		Flat B		Flat C		
			Water	Oyster	Water	Oyster	Water	Oyster	
12-25-55	7:00 a.m.			<18		<18		<18	
12-26-55	8:15 a.m.			<18		<18		<18	
	10:30 a.m.	<0.41	1100		130		<1.8		1100
	5:45 p.m.	<0.41	1400		130		<1.8		1300
12-27-55	7:00 a.m.	<0.41	1700	7900	70	490	<1.8	<18	1100
	10:00 a.m.		790	24000	130	1300	<1.8	<18	
	2:00 p.m.	<0.41	1100	7900	49	490	<1.8	<18	
	6:00 p.m.	<0.41	1300	17000	79	790	<1.8	<18	1600
	9:00 p.m.		2400	7900	130	2400	<1.8	<18	
12-28-55	7:00 a.m.	<0.41	3500	4900	70	790	2.0	<18	1000
	10:00 a.m.		1300	9500	49	790	<1.8	<18	
	2:00 p.m.	<0.41	1300	4900	79	330	<1.8	<18	
	6:00 p.m.	<0.41	790	7900	79	230	<1.8	<18	1200
	9:00 p.m.		790	11000	33	490	<1.8	<18	
12-29-55	7:00 a.m.	<0.41	1700	13000	130	490	<1.8	<18	1200
	10:00 a.m.		3500	7000	350	330	<1.8	<18	
	2:00 p.m.	<0.41	1700	11000	49	700	<1.8	<18	
	6:00 p.m.	<0.41	1700	1700	49	790	<1.8	<18	
	9:00 p.m.		1400	7900	79	330	<1.8	<18	

TABLE 17. RELATIVE COLIFORM CONTENT OF OYSTERS AND OVERLYING WATER

Run No. 17 - January 8 to January 12, 1956

Date	Time	U.V. Eff.	Coliform M.P.N. per 100 ml						Estimated coliforms added to flat A (Per 100 ml.)
			Flat A		Flat B		Flat C		
			Water	Oyster	Water	Oyster	Water	Oyster	
1-8-56	8:00 a.m.			<18		<18		<18	
1-9-56	8:00 a.m.			<18		<18		<18	
	10:30 a.m.		5400		23		<1.8		1000
	2:00 p.m.	<0.41							
	6:00 p.m.	<0.41	2200		70		<1.8		1100
1-10-56	7:00 a.m.	<0.41	1100	17000	130	330	<1.8	<18	1000
	10:00 a.m.		790	3500	79	790	<1.8	<18	
	2:00 p.m.	<0.41	790	17000	49	790	<1.8	<18	
	6:00 p.m.	<0.41	1100	3300	79	490	<1.8	<18	900
	9:00 p.m.		1300	24000	49	230	<1.8	<18	
1-11-56	7:00 a.m.	<0.41	1100	7000	33	230	1.8	68	
	10:00 a.m.		2400	4900	49	220	<1.8	<18	
	2:00 p.m.	<0.41	2400	22000	79	330	<1.8	<18	
	6:00 p.m.	<0.41	490	2300	33	790	<1.8	<18	1000
	9:00 p.m.		1400	3300	33	490	<1.8	<18	
1-12-56	7:00 a.m.	<0.41	700	7900	33	330	<1.8	<18	960
	10:00 a.m.		1300	2300	33	490	<1.8	<18	
	2:00 a.m.	<0.41	1100	13000	49	230	<1.8	<18	
	6:00 p.m.	<0.41	2200	2300	79	230	<1.8	<18	
	9:00 p.m.		490	24000	70	78	<1.8	<18	

TABLE 18. RELATIVE COLIFORM CONTENT OF OYSTERS AND OVERLYING WATER

Run No. 18 - February 12 to February 16, 1956

Date	Time	U.V. Eff.	Coliform M.P.N. per 100 ml						Estimated coliforms added to flat A (Per 100 ml.)
			Flat A		Flat B		Flat C		
			Water	Oyster	Water	Oyster	Water	Oyster	
2-12-56				<18		<18		<18	
2-13-56	8:30 a.m.			20		20		20	
	11:00 a.m.		1300		49		<1.8		1200
	2:00 p.m.	<0.41							
	6:00 p.m.	<0.41	5400		240		<1.8		1300
2-14-56	7:00 a.m.	<0.41	790	4900	79	140	<1.8	<18	1200
	10:00 a.m.		330	4600	170	1300	<1.8	<18	
	2:00 p.m.	<0.41	1300	4900	70	230	<1.8	<18	
	6:00 p.m.	<0.41	790	3300	49	2400	<1.8	<18	
	9:00 p.m.		790	3300	49	330	<1.8	<18	
2-15-56	7:00 a.m.	<0.41	1300	7900	130	310	<1.8	<18	1200
	10:00 a.m.		490	3300	79	490	<1.8	<18	
	2:00 p.m.	<0.41	1300	3300	79	330	<1.8	<18	
	6:00 p.m.	<0.41	790	2300	240	790	<1.8	<18	
	9:00 p.m.		230	4900	130	490	<1.8	<18	
2-16-56	7:00 a.m.	<0.41	790	3300	49	330	<1.8	<18	
	10:00 a.m.	<0.41	330	7900	70	790	<1.8	<18	
	2:00 p.m.		1300	17000	79	490	<1.8	<18	
	6:00 p.m.		700	11000	70	490	<1.8	<18	
	9:00 p.m.		1300	11000	130	490	<1.8	<18	